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(71) Applicant: **Beijing Tide Pharmaceutical Co., Ltd.**
Beijing 100176 (CN)

(72) Inventors:
- **ZHAO, Yanping**
  Beijing 100176 (CN)
- **WANG, Hongjun**
  Beijing 100176 (CN)
- **JIANG, Yuanyuan**
  Beijing 100176 (CN)
- **ZHONG, Weiting**
  Beijing 100176 (CN)
- **PANG, Jianmei**
  Beijing 100176 (CN)
- **LI, Gong**
  Beijing 100176 (CN)
- **LI, Xiang**
  Beijing 100176 (CN)
- **HE, Yixin**
  Beijing 100176 (CN)
- **ZHOU, Liying**
  Beijing 100176 (CN)
- **LIU, Yanan**
  Beijing 100176 (CN)

(74) Representative: **Turner, Craig Robert et al**
A.A. Thornton & Co.
10 Old Bailey
London EC4M 7NG (GB)

(54) DOUBLE-STRANDED RNA MOLECULE TARGETING CKIP-1 AND USE THEREOF

(57) The present invention relates to the field of biomedicine, particularly to double-stranded RNA molecules targeting CKIP-1 and uses thereof, particularly to use of the double-stranded RNA molecules for the treatment of inflammatory diseases such as arthritis, particularly rheumatoid arthritis.

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to the field of biomedicine, particularly to double-stranded RNA molecules targeting CKIP-1 and uses thereof, particularly to use of the double-stranded RNA molecules for the treatment of inflammatory diseases such as arthritis, particularly rheumatoid arthritis.

BACKGROUND OF THE INVENTION

[0002] Casein Kinase Interacting Protein 1 (CKIP-1) is a bone formation inhibiting gene that specifically regulates bone formation rather than bone resorption. CKIP-1 is highly expressed in bone tissue of patients with osteoporosis. Targeted inhibition of CKIP-1 expression has been proven to be useful in the treatment of osteoporosis or other pathological bone destruction. However, CKIP-1 has not been associated with inflammation in the art.

[0003] TNF-α and IL-6 are two important pro-inflammatory cytokines and play an important role in the inflammatory response of the body. The levels of TNF-α and IL-6 are low in human bodies under physiological conditions. However, under pathological conditions, increased secretion of TNF-α and IL-6, and the resulting cascade of various pro-inflammatory factors, can lead to an inflammatory response, and thus tissue damage. Inflammatory diseases have been treated in the art by targeted inhibition of TNF-α and IL-6. For example, a number of inhibitors targeting TNF-α have been marketed, including Infliximab, Etanercept, Adalimumab, Golimumab, and Certolizumab. In addition, IL-6 blockers have been marketed for clinical use, such as tolizumab. In a large randomized, double-blind clinical trial, tolizumab has a good therapeutic effect in patients who do not respond to TNF-α mAb.

[0004] Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by multi-joint synovitis. Prolonged recurrent episodes of synovitis can lead to destruction of intra-articular cartilage and bone, joint dysfunction, and even disability. Rheumatoid arthritis has a high incidence in adults, about 20-40% per 100,000 adults. Studies have shown that 70-75% of rheumatoid arthritis patients have bone destruction within 3 years of onset, 10% of them have severe dysfunction within 2 years of onset, and about 50% of them lose their ability to work after 10 years of onset, resulting in serious economic burden to both patients and society. At present, the drugs for treating RA mainly comprise non-steroidal anti-inflammatory drugs, hormones, anti-rheumatic drugs and the like, and are mainly used for relieving pain, relieving inflammation but are not effective in preventing joint and bone destruction. In recent years, some new biological agents can alleviate and inhibit the occurrence of bone destruction, but cannot repair the existing bone injury. There is currently a clinical lack of RA therapeutics that both reduce inflammation and promote bone repair.

SUMMARY OF THE INVENTION

[0005] In a first aspect, the present invention provides a double-stranded RNA (dsRNA) molecule comprising a sense strand and an antisense strand which are selected from the group consisting of:

1) a sense strand shown in SEQ ID NO: 63 and an antisense strand shown in SEQ ID NO: 64;
2) a sense strand shown in SEQ ID NO: 71 and an antisense strand shown in SEQ ID NO: 72;
3) a sense strand shown in SEQ ID NO: 83 and an antisense strand shown in SEQ ID NO: 84; and
4) a sense strand shown in SEQ ID NO: 161 and an antisense strand shown in SEQ ID NO: 162.

[0006] In some embodiments, the sense and/or antisense strand additionally has an overhang of at least one nucleotide at the 3' end. In some embodiments, the sense and/or antisense strand additionally has an overhang of 2 nucleotides at the 3' end, preferably the overhang is TT.

[0007] In some embodiments, the sense and antisense strands comprise 1 or 2 nucleotide substitutions located within the 6, 5, 4, 3, or 2 nucleotides from the 5' and/or 3' end. In some embodiments, the sense and antisense strands comprise 1 nucleotide substitution located at the last nucleotide of the 3' end of the sense strand and correspondingly at the first nucleotide of the 5' end of the antisense strand.

[0008] In some embodiments, the dsRNA comprises at least one modified nucleotide. In some embodiments, the modified nucleotide is selected from the group consisting of: 2'-O-methyl modified nucleotides, 2'-F modified nucleotides, nucleotides containing 5'-phosphorothioate groups and end nucleotides linked to cholesteryl derivatives or dodecanoic acid bisdecylamide groups, 2'-deoxy-2'-fluoro modified nucleotides, 2'-deoxy-modified nucleotides, locked nucleotides, abasic nucleotides, 2'-amino-modified nucleotides, 2'-alkyl-modified nucleotides, morpholino nucleotides, phosphoramidates and nucleotides containing non-natural bases. In some embodiments, the 2' hydroxyl groups of all nucleotides with uracil or cytosine bases in the sense and/or antisense strands of the dsRNA are modified with methoxy groups.

[0009] In some embodiments, the dsRNA molecule is an siRNA or shRNA. In some embodiments, the dsRNA molecule inhibits CKIP-1 expression by at least 50%, preferably by at least 70%. In some embodiments, the dsRNA molecule inhibits expression of a pro-inflammatory cytokine such as IL-6, TNF-α, and/or IL-17A.

[0010] In a second aspect, the invention also provides an expression vector comprising a nucleotide sequence encoding the dsRNA molecule of the invention, and the nucleotide sequence is operably linked to a transcription regulation element.

[0011] In a third aspect, the invention also provides a pharmaceutical composition comprising the dsRNA molecule of the invention or the expression vector of the invention, and a pharmaceutically acceptable carrier.

[0012] In a fourth aspect, the invention provides a method of treating arthritis, particularly rheumatoid arthritis, in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the dsRNA molecule of the invention or the expression vector of the invention or the pharmaceutical composition of the invention. In some embodiments, the method further comprises administering to the subject an additional therapeutic agent for treating arthritis, particularly rheumatoid arthritis.

[0013] In a fifth aspect, the invention provides the use of the dsRNA molecule of the invention or the expression vector of the invention or the pharmaceutical composition of the invention for the manufacture of a medicament for the treatment of arthritis, in particular rheumatoid arthritis, in a subject in need thereof.

[0014] In a sixth aspect, the invention provides a method of treating an inflammatory disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the dsRNA molecule of the invention or the expression vector of the invention or the pharmaceutical composition of the invention. In some embodiments, the method further comprises administering to the subject an additional therapeutic agent for treating an inflammatory disease.

[0015] In a seventh aspect, the invention provides the use of the dsRNA molecule of the invention or the expression vector of the invention or the pharmaceutical composition of the invention in the preparation of a medicament for treating an inflammatory disease in a subject in need thereof.

[0016] In an eighth aspect, the present invention provides a method of treating a bone metabolism-related disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the dsRNA molecule of the present invention or the expression vector of the present invention or the pharmaceutical composition of the present invention. In some embodiments, the method further comprises administering to the subject an additional therapeutic agent for treating a bone metabolism-related disorder.

[0017] In a ninth aspect, the invention provides the use of the dsRNA molecule of the invention or the expression vector of the invention or the pharmaceutical composition of the invention in the preparation of a medicament for treating a bone metabolism-related disorder in a subject in need thereof.

[0018] In various aspects of the invention, the subject is a human.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019]

Fig. 1 shows a pool of candidate siRNA sequences, and the TT at the 3' end of each sequence is the overhang which is not complementary to the target sequence.
Fig. 2 shows the map of an overexpression vector for dual luciferase assay.
Fig. 3 shows the inhibitory effect of si-TD137 on CKIP-1 expression in a dual luciferase assay.
Fig. 4 shows the inhibitory effect of si-TD141 on CKIP-1 expression in a dual luciferase assay.
Fig. 5 shows the inhibitory effect of si-TD176 on CKIP-1 expression in a dual luciferase assay.
Fig. 6 shows the inhibitory effect of si-7 on CKIP-1 expression in a dual luciferase assay.
Fig. 7 shows that siRNAs decrease CIA mouse clinical score.
Fig. 8 shows body weight changes in CIA mice after siRNA treatment.
Fig. 9 shows that siRNAs affect proinflammatory cytokine expression in joint tissue of CIA mice.

DETAILED DESCRIPTION OF THE INVENTION

I. Definition

[0020] In the present invention, unless otherwise indicated, scientific and technical terms used herein have the meaning commonly understood by those skilled in the art. Moreover, the terms related to protein and nucleic acid chemistry, molecular biology, cell and tissue culture, microbiology, immunology, and laboratory procedures and routine procedures used herein are terms and procedures widely used in the relevant fields. Meanwhile, in order to better understand the present invention, definitions and explanations of related terms are provided below.

[0021] Unless otherwise indicated, the nucleic acid sequences recited herein are written in a direction from 5' to 3'. The term "nucleic acid" refers to DNA or RNA or modified forms thereof comprising purine or pyrimidine bases present in DNA (adenine "A", cytosine "C", guanine "G", thymine "T") or purine or pyrimidine bases present in RNA (adenine "A", cytosine "C", guanine "G", uracil "U"). The double-stranded RNA nucleic acid molecules provided herein may also comprise a "T" base at the 3' end, even if the "T" base is not naturally present in an RNA. In some cases, these bases may be denoted as "dT" to distinguish deoxyribonucleotides present in the ribonucleotide chain.

[0022] When a nucleic acid molecule selectively reduces or inhibits the expression of a gene, the gene is "targeted" by the nucleic acid molecule described herein. Alternatively, when a nucleic acid molecule hybridizes under stringent conditions to a transcript of a gene (i.e., mRNA thereof), the nucleic acid molecule targets the gene. Being capable of hybridizing "under stringent conditions" means annealing to the target mRNA region under standard conditions that tend to be detrimental to hybridization, e.g., high temperature and/or low salt content. Suitable processes, including 0.1 x SSC, 68°C, 2 hours, are described in Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1982.

[0023] As used herein, "CKIP-1" refers to the CKIP-1 gene or protein (also known as PLEKHO1). Examples of the sequences of CKIP-1 include, but are not limited to: human: Genbank number NM_016274.4; mouse: Genbank number NM_023320.2; rat: Genbank number NM_001025119.1 and cynomolgus monkey: Genbank numbers XM001098879 and XM001098774.

[0024] As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during transcription of a CKIP-1 gene, including mRNA as an RNA processing product of a primary transcript.

[0025] As used herein and unless otherwise indicated, the term "complementary", when used to describe a relationship between a first nucleotide sequence and a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize under specific conditions to an oligonucleotide or polynucleotide comprising the second nucleotide sequence and form a duplex structure, as will be understood by those skilled in the art. For example, such conditions can be stringent conditions, wherein the stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA at 50 °C or 70 °C for 12-16 hours followed by washing. Other conditions may also be used, such as physiologically relevant conditions that may be encountered in an organism. Those skilled in the art will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

[0026] This includes base pairing of an oligonucleotide or polynucleotide comprising the first nucleotide sequence with an oligonucleotide or polynucleotide comprising the second nucleotide sequence over the full length of the first and second nucleotide sequences. These sequences may be referred to herein as being "completely complementary" to each other. However, when reference is made herein to the first sequence being " substantially complementary" to the second sequence, the two sequences may be completely complementary or form one or more, but typically no more than 4, 3 or 2, mismatched base pairs upon hybridization, while retaining the ability to hybridize under the conditions most relevant to their ultimate application. However, when two oligonucleotides are designed to form one or more single stranded overhangs upon hybridization, such overhangs should not be considered mismatches when referring to the definition of complementarity. For example, in a dsRNA comprising one oligonucleotide of 19 nucleotides in length and another oligonucleotide of 21 nucleotides in length, the longer oligonucleotide comprises a sequence of 19 nucleotides that is fully complementary to the shorter oligonucleotide, which may also be referred to as being "completely complementary".

[0027] "Complementary" sequences, as used herein, may also comprise or be entirely formed from non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, as long as the above requirements regarding their ability to hybridize are met. These non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogstein base pairing.

[0028] As used herein for base matching between the sense strand and the antisense strand of a dsRNA, or base matching between the antisense strand of a dsRNA and the target sequence, the terms "complementary", "fully complementary" and "substantially complementary" may be used, which are to be understood in accordance with the context.

[0029] As used herein, a polynucleotide that is "substantially complementary to at least a portion of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of a target mRNA (e.g., an mRNA encoding CKIP-1) that includes a 5' UTR, an open reading frame (ORF), or a 3' UTR. For example, a polynucleotide is complementary to at least a portion of CKIP-1 if its sequence is substantially complementary to a non-interrupted portion of an mRNA encoding CKIP-1.

[0030] Recently, it has been found that double-stranded RNA molecules (dsRNA) block gene expression through a highly conserved regulatory mechanism known as RNA interference (RNAi). WO 99/32619 (Fire et al.) discloses the use of dsRNA of at least 25 nucleotides in length to inhibit C. elegans gene expression. dsRNA has also been found to degrade target RNA in other organisms including plants (see, e.g., WO 99/53050, Waterhouse et al., and WO 99/61631, Heifetz et al.), Drosophila (see, e.g., Yang, D., et al., Curr. Biol. (2000) 10: 1191-1200), and mammals (see WO 00/44895, Limmer and DE 10100586.5, Kreutzer et al.). This natural mechanism has become a hot spot in the development of new drugs for the treatment of diseases caused by abnormal or harmful regulation of genes.

[0031] As used herein, the term "double-stranded RNA" or "dsRNA" refers to a duplex structure comprising two antiparallel and substantially complementary nucleic acid strands as described above. Typically, the majority of the nucleotides of each strand are ribonucleotides, but as detailed herein, each strand or both strands may also comprise at least one non-ribonucleotide, such as a deoxyribonucleotide and/or a modified nucleotide. In addition, "dsRNA" as used in this specification may include chemical modifications to ribonucleotides, including modifications at multiple nucleotides, and includes all types of modifications disclosed herein or known in the art.

[0032] The two strands forming the duplex structure may be different portions of the same larger RNA molecule, or they may be separate RNA molecules. If the two strands are separate RNA molecules, such dsRNA is often referred to in the literature as siRNA ("short interfering RNA"). If the two strands are parts of a larger molecule and are joined by a non-interrupted nucleotide strand between the 3'-end of one strand and the 5'-end of the other strand forming the duplex structure, the joined RNA strand is referred to as a "hairpin loop", a "short hairpin RNA", or a "shRNA". If the two strands are covalently linked by means other than a non-interrupted strand between the 3'-end of one strand and the 5'-end of the other strand forming a duplex structure, the linkage structure is referred to as a "linker". The RNA strands may have the same or different number of nucleotides. In addition to the duplex structure, the dsRNA may comprise overhangs of one or more nucleotides. Typically, most of the nucleotides of each strand are ribonucleotides, but as described in detail herein, each strand or both strands may also comprise at least one non-ribonucleotide, for example, a deoxyribonucleotide and/or a modified nucleotide.

[0033] As used herein, "overhang" refers to one or more unpaired nucleotides that protrude from the duplex structure of a dsRNA when the 3' end of one strand of the dsRNA extends beyond the 5' end of the other strand or vice versa. "Blunt end" or "blunt-ended" means that there are no unpaired nucleotides at the end of the dsRNA, i.e., there are no nucleotide overhangs. A "blunt-ended" dsRNA refers to a dsRNA that is double-stranded over its entire length, i.e., without nucleotide overhang at either end of the molecule. For clarity, chemical caps or non-nucleotide chemical moieties coupled to the 3'-or 5'-ends of the dsRNA are not considered in determining whether the dsRNA has overhangs or blunt ends.

[0034] The term "antisense strand" refers to a strand of dsRNA comprising a region substantially complementary to a target sequence. As used herein, the term "complementary region" refers to a region of the antisense strand that is substantially complementary to a sequence as defined herein (e.g., a target sequence). If the complementary region is not fully complementary to the target sequence, the mismatch may be located in an internal or end region of the molecule. Typically, the most tolerable mismatch is located within the terminal regions (excluding overhangs described herein), e.g., within 6, 5, 4, 3 or 2 nucleotides from the 5' and/or 3' ends, or the last 1 nucleotide at the 5' and/or 3' ends.

[0035] As used herein, the term "sense strand" refers to a strand of dsRNA comprising a region substantially complementary to the region of the antisense strand.

[0036] The term "subject" or "individual" as used herein means a mammal, particularly a primate, particularly a human.

[0037] As used herein, "treating" an individual suffering from a disease or disease condition means that the individual's symptoms are partially or completely alleviated, or remain unchanged after treatment. Thus, treatment includes prevention, treatment and/or cure. Prevention refers to prevention of a potential disease and/or prevention of worsening of symptoms or disease progression. Treatment also includes any pharmaceutical uses of any dsRNA, expression vector, and composition provided herein.

[0038] As used herein, "therapeutic effect" means an effect resulting from treatment of an individual that alters, generally ameliorates or alleviates the symptoms of the disease or disease condition, or cures the disease or disease condition.

[0039] As used herein, "therapeutically effective amount" or "therapeutically effective dose" refers to an amount of a substance, compound, material, or composition comprising a compound that is at least sufficient to produce a therapeutic effect after administration to a subject. Thus, it is the amount necessary to prevent, cure, ameliorate, arrest or partially arrest the symptoms of the disease or condition. For example, if a given clinical treatment that decreases a measurable parameter associated with a disease or condition by at least 25% is considered to be an effective treatment, a therapeutically effective amount of the drug used to treat the disease or condition is the amount necessary to decrease the parameter by at least 25%.

[0040] The term "pharmaceutically acceptable carrier" refers to a carrier used to administer a therapeutic agent (e.g., dsRNA). Such carriers include, but are not limited to, saline, buffered saline solution, glucose, water, glycerol, ethanol, and combinations thereof.

[0041] As used herein, an "expression vector" includes a vector capable of expressing a nucleotide sequence of interest operably linked to regulatory sequences, such as promoter regions, capable of affecting expression of such nucleotide sequence. Such additional fragments may include promoter and terminator sequences, and optionally may include one or more origins of replication, one or more selectable markers, enhancers, polyadenylation signals, and the like.

[0042] As used herein, "operably linked "with respect to a nucleic acid sequence, region, element, or domain means that the nucleic acid regions are functionally related to each other. For example, a promoter may be operably linked to a nucleotide sequence encoding a dsRNA such that the promoter regulates or mediates transcription of the nucleotide sequence.

II. Nucleic acid molecules targeting CKIP-1

[0043] The present inventors designed, synthesized and screened out dsRNA molecules capable of significantly inhibiting CKIP-1 expression. Surprisingly, the dsRNA molecules as obtained can both reduce inflammation and promote bone repair, and thus can be effectively used for treating arthritis, such as rheumatoid arthritis (RA).

[0044] In one aspect, the invention provides a nucleic acid molecule targeting CKIP-1, such as a dsRNA molecule, which comprises a sense strand and a corresponding complementary antisense strand selected from Table 1.

[0045] In some preferred embodiments, the CKIP-1-targeting nucleic acid molecule comprises a sense and an antisense strand corresponding to si-TD060, si-TD062, si-TD066, si-TD068, si-TD070, si-TD074, si-TD080, si-TD082, si-TD089, si-TD096, si-TD137, si-TD140, si-TD141, si-TD143, si-TD176, si-TD178, si-TD181, si-TD362, si-TD364, si-TD378, si-TD726, si-TD730, si-7, si-10 in Table 1.

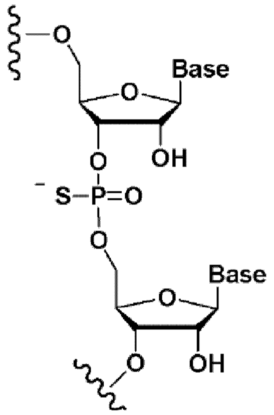
[0046] In some more preferred embodiments, the nucleic acid molecule targeting CKIP-1 comprises a sense strand and an antisense strand corresponding to si-TD137, si-TD141, si-TD176, si-7 in Table 1.

[0047] In some embodiments, the sense strand and/or the antisense strand of the nucleic acid molecule additionally has an overhang of at least one nucleotide at the 3' end. For example, the sense and/or antisense strand additionally has an overhang of 1, 2 or 3 nucleotides at the 3' end. For example, in some embodiments, the overhang is TT (i.e., dTdT). In some embodiments, the sense and antisense strands of the nucleic acid molecule comprise an additional overhang TT at the 3' end.

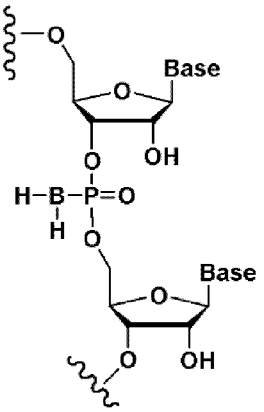
[0048] In some embodiments, the sense strand and/or the antisense strand in the nucleic acid molecule comprises at least 1, e.g., 1 or 2 nucleotide substitutions. For example, the substitution is located within 6, 5, 4, 3 or 2 nucleotides from the 5' and/or 3' ends. In some embodiments, the sense and antisense strands of the nucleic acid molecule comprise 1 nucleotide substitution at the 3' last nucleotide position of the sense strand and correspondingly at the 5' first nucleotide position of the antisense strand. Such substitutions may result in mismatches with the target sequence, however mismatches as defined herein are tolerated, without significantly affecting or without affecting the activity of the dsRNA.

[0049] In some embodiments, the dsRNA of the present invention comprises at least one modified nucleotide. The modified nucleotide may comprise modification of the phosphate group, the ribose group and/or the base group.

[0050] For example, modification of the phosphate group in a nucleotide includes modification of the oxygen in the phosphate group, such as phosphorothioate modification and boranophosphate modification. The oxygen in the phosphate group is substituted with sulfur and borane, respectively, as shown in the following formula. Both modifications stabilize the nucleic acid structure and maintain high specificity and affinity for base pairing.

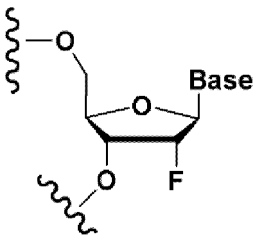


Phosphorothioate modification

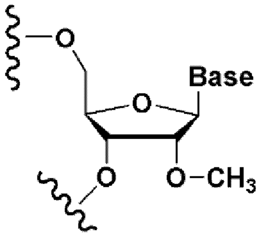


Borylation phosphate modification

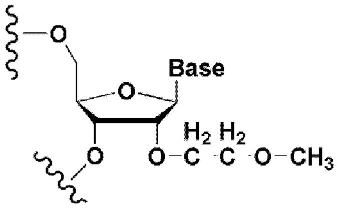
[0051] Modification of the ribose group in a nucleotide includes modification of the 2'-hydroxyl group (2'-OH) in the ribose group. During RNA hydrolysis, under the catalysis of RNase, 2'-OH first attacks the phosphate group, forms a cyclic phosphodiester while breaking a phosphate ester bond, and then forms the hydrolysates under the action of alkali. If certain substituents such as methoxy group or fluorine group are introduced into the 2'-hydroxyl position of the ribose group, the nucleic acid such as siRNA may have stronger nuclease hydrolysis resistance, and the stability of the nucleic acid is improved. Modifications to the 2'-hydroxyl group of the nucleotide pentose include, but are not limited to, 2'-fluoro modification, 2'-methoxy modification (2'-OME), 2'-methoxyethyl modification (2'-MOE), 2'-2,4-dinitrophenol modification (2'-DNP modification), Locked nucleic acid modification (LNA modification), 2'-Amino modification, 2'-Deoxy modification, 3'-Cholesterol modification, 4'-thiothymidine modification, and the like. Examples of structures for such modifications are as follows:



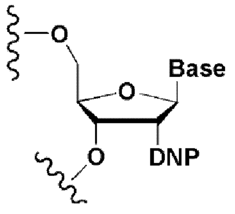
2'-fluoro modification



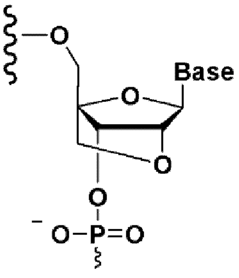
2'-methoxy modification



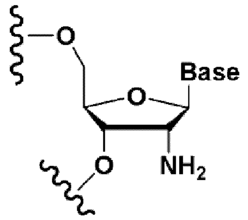
2'-methoxyethyl modification



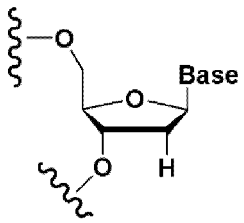
2'-2, 4-dinitrophenol modification



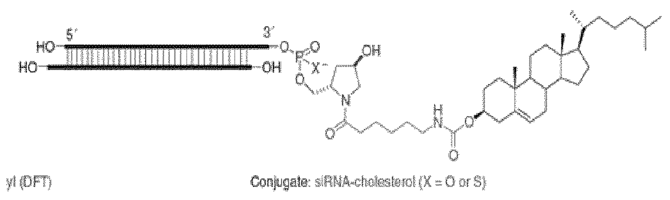
locked nucleic acid modification



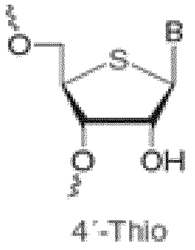
2'-amino modification



2'-deoxy modification



3'-cholesterol modification



4'-thiodeoxythymidine modification

[0052] The modification of the base group in the nucleotide refers to modifying the base in the nucleotide group to enhance the interaction between bases, so as to improve the effect on the target mRNA. For example, 5'-bromouracil and 5'-iodouracil modifications, which introduce bromine or iodine at the 5' position of uracil, are commonly used base modifications. Other modifications include N3-methyl-uracil modification, 2,6-diaminopurine modification, etc.

[0053] In some embodiments, the dsRNA of the present invention comprises at least one modified nucleotide selected from the group consisting of: 2'-O-methyl modified nucleotides, 2'-F modified nucleotides, nucleotides comprising a 5'-phosphorothioate group, and end nucleotides linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, and/or, for example, the modified nucleotides are selected from the group consisting of: 2'-deoxy-2'-fluoro modified nucleotides, 2'-deoxy-modified nucleotides, locked nucleotides, abasic nucleotides, 2'-amino-modified nucleotides, 2'-alkyl-modified nucleotides, morpholino nucleotides, phosphoramidates, and nucleotides containing unnatural bases. The at least one modified nucleotide may, for example, enhance the stability of the dsRNA and/or reduce the immunogenic effect of the dsRNA. The modified nucleotides may be on the sense strand and/or on the antisense strand.

[0054] In some embodiments, the dsRNA comprises at least one 2'-O-methyl modified ribonucleotide and/or at least one nucleotide comprising a 5'-phosphorothioate group.

[0055] In some specific embodiments, the 2' hydroxyl groups of all nucleotides with uracil or cytosine bases in the sense and/or antisense strands of the dsRNA of the present invention are modified with methoxy groups.

[0056] In some embodiments, the 2' hydroxyl groups of all nucleotides with uracil or cytosine bases in the sense strand of the dsRNA of the present invention are modified with methoxy groups.

[0057] In some embodiments, the 2' hydroxyl groups of all nucleotides in the sense and/or antisense strands of the dsRNA of the present invention are modified with methoxy groups.

[0058] In some embodiments, the 2' hydroxyl groups of all nucleotides in the sense strand of the dsRNA of the invention are modified with methoxy groups.

[0059] In some embodiments, the 5' end of the sense strand and/or the antisense strand of the dsRNA of the invention is phosphorylated.

[0060] In some embodiments, the sense strand and/or the antisense strand of the dsRNA of the present invention comprises a 3' cholesterol modification.

[0061] In some embodiments, the 2' hydroxyl groups of all the nucleotides with uracil bases or cytosine bases in the sense strand of the dsRNA of the present invention are modified with fluorine (F).

[0062] In some embodiments, the dsRNA of the invention comprises a locked nucleic acid modification in the sense strand.

[0063] In some embodiments, all nucleotides in the sense strand and/or antisense strand of the dsRNA of the invention comprise phosphorothioate modifications.

[0064] In some embodiments, the dsRNA molecule is an siRNA.

[0065] In still other embodiments, the dsRNA molecule is shRNA (short hairpin RNA). It is within the ability of those skilled in the art to design suitable shRNAs based on siRNA sequences.

[0066] The dsRNA of the present invention may be obtained by conventional techniques in the art such as solid phase synthesis or liquid phase synthesis. Modified nucleotides can be introduced by using modified nucleotide monomers during the synthesis.

[0067] In yet another aspect, the invention provides an expression vector comprising a nucleotide sequence encoding a nucleic acid molecule of the invention, such as dsRNA, wherein the nucleotide sequence is operably linked to a transcription regulatory element, such as a promoter or the like. Recombinant vector capable of expressing a dsRNA molecule can be delivered to and permanently present in the target cells. Alternatively, a vector providing transient expression of the nucleic acid molecule may be used. If desired, the vector may be administered repeatedly. Once expressed, the dsRNA molecule interacts with the target mRNA and generates an RNA interference response. In general, shRNAs are particularly suitable for being produced in this manner.

[0068] The expression vector may be a linear construct, a circular plasmid vector, or a viral vector (including but not limited to adenovirus, adeno-associated virus, lentiviral vector, etc.). In the case of siRNA, individual strands of siRNA can be transcribed from promoters on two separate expression vectors; alternatively, individual strands of siRNA may be transcribed from promoters both located on the same expression plasmid. In the case of shRNA, the shRNA strand is transcribed from a single expression vector.

[0069] The promoter driving dsRNA expression in the expression vector of the present invention may be eukaryotic RNA polymerase I promoter (e.g., ribosomal RNA promoter), RNA polymerase II promoter (e.g., CMV early promoter or actin promoter or UlsnRNA promoter) or generally RNA polymerase III promoter (e.g., U6snRNA or 7SKRNA promoter) or prokaryotic promoter (e.g., T7 promoter, provided that the expression vector also encodes the T7 RNA polymerase required for transcription from the T7 promoter).

[0070] The dsRNA of the present invention can significantly inhibit the expression of CKIP-1 in cells. In some embodiments, expression of CKIP-1 is inhibited by at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or even 100%. Preferably, the dsRNA of the present invention is capable of inhibiting CKIP-1 expression by at least 50%. More preferably, the dsRNA of the present invention is capable of inhibiting CKIP-1 expression by at least 70%.

[0071] When the terms "inhibit the expression of', "downregulate the expression of', "suppress the expression of', and the like are used in reference to a CKIP-1 gene, they refer herein to the at least partial inhibition of the expression of the CKIP-1 gene, as manifested by a decrease in the level of CKIP-1 expression in a first cell or group of cells in which the CKIP-1 gene is transcribed and which has or have been treated such that the expression of the CKIP-1 gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). The degree of inhibition is usually expressed in the following way:

(CKIP-1 expression level in control cells - CKIP-1 expression level in treated cells)/ CKIP-1 expression level in control cells x 100%.

[0072] The expression level may be an mRNA level or a protein level. It is clear to those skilled in the art how to determine the mRNA level or the corresponding protein level of a particular gene.

[0073] Surprisingly, the dsRNA of the present invention may also inhibit the expression of the pro-inflammatory cytokines IL-6, TNF-α and/or IL-17A. In particular, the dsRNA of the present invention can significantly inhibit the expression of the pro-inflammatory cytokine IL-6.

[0074] In some embodiments, expression of the pro-inflammatory cytokines IL-6, TNF-α, and/or IL-17A is inhibited by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or even 100%. In some preferred embodiments, the expression of IL-6 is inhibited by at least 50%, more preferably by at least 80%.

Table 1 dsRNA inhibiting CKIP-1 expression

siRNA	Sequence (5'-3')		SEQ ID NO
si-TD037	Sense strand	AAGGUCGGCUGGGUCCGGA	1
	Antisense strand	UCCGGACCCAGCCGACCUU	2
si-TD040	Sense strand	GUCGGCUGGGUCCGGAAAU	3
	Antisense strand	AUUUCCGGACCCAGCCGAC	4
si-TD042	Sense strand	CGGCUGGGUCCGGAAAUUC	5
	Antisense strand	GAAUUUCCGGACCCAGCCG	6
si-TD044	Sense strand	GCUGGGUCCGGAAAUUCUG	7
	Antisense strand	CAGAAUUUCCGGACCCAGC	8
si-TD050	Sense strand	UCCGGAAAUUCUGCGGGAA	9
	Antisense strand	UUCCCGCAGAAUUUCCGGA	10
si-TD057	Sense strand	AUUCUGCGGGAAAGGGAUU	11
	Antisense strand	AAUCCCUUUCCCGCAGAAU	12
si-TD058	Sense strand	UUCUGCGGGAAAGGGAUUU	13
	Antisense strand	AAAUCCCUUUCCCGCAGAA	14
si-TD060	Sense strand	CUGCGGGAAAGGGAUUUUC	15
	Antisense strand	GAAAAUCCCUUUCCCGCAG	16
si-TD061	Sense strand	UGCGGGAAAGGGAUUUUCA	17
	Antisense strand	UGAAAAUCCCUUUCCCGCA	18
si-TD062	Sense strand	GCGGGAAAGGGAUUUUCAG	19
	Antisense strand	CUGAAAAUCCCUUUCCCGC	20
si-TD064	Sense strand	GGGAAAGGGAUUUUCAGGG	21
	Antisense strand	CCCUGAAAAUCCCUUUCCC	22
si-TD065	Sense strand	GGAAAGGGAUUUUCAGGGA	23
	Antisense strand	UCCCUGAAAAUCCCUUUCC	24
si-TD066	Sense strand	GAAAGGGAUUUUCAGGGAG	25
	Antisense strand	CUCCCUGAAAAUCCCUUUC	26
si-TD067	Sense strand	AAAGGGAUUUUCAGGGAGA	27
	Antisense strand	UCUCCCUGAAAAUCCCUUU	28
si-TD068	Sense strand	AAGGGAUUUUCAGGGAGAU	29
	Antisense strand	AUCUCCCUGAAAAUCCCUU	30
si-TD070	Sense strand	GGGAUUUUCAGGGAGAUUU	31
	Antisense strand	AAAUCUCCCUGAAAAUCCC	32

(continued)

siRNA	Sequence (5’-3’)		SEQ ID NO
si-TD072	Sense strand	GAUUUUCAGGGAGAUUUGG	33
	Antisense strand	CCAAAUCUCCCUGAAAAUC	34
si-TD074	Sense strand	UUUUCAGGGAGAUUUGGAA	35
	Antisense strand	UUCCAAAUCUCCCUGAAAA	36
si-TD076	Sense strand	UUCAGGGAGAUUUGGAAAA	37
	Antisense strand	UUUUCCAAAUCUCCCUGAA	38
si-TD078	Sense strand	CAGGGAGAUUUGGAAAAAC	39
	Antisense strand	GUUUUUCCAAAUCUCCCUG	40
si-TD080	Sense strand	GGGAGAUUUGGAAAAACCG	41
	Antisense strand	CGGUUUUUCCAAAUCUCCC	42
si-TD082	Sense strand	GAGAUUUGGAAAAACCGCU	43
	Antisense strand	AGCGGUUUUUCCAAAUCUC	44
si-TD084	Sense strand	GAUUUGGAAAAACCGCUAU	45
	Antisense strand	AUAGCGGUUUUUCCAAAUC	46
si-TD087	Sense strand	UUGGAAAAACCGCUAUGUG	47
	Antisense strand	CACAUAGCGGUUUUUCCAA	48
si-TD089	Sense strand	GGAAAAACCGCUAUGUGGU	49
	Antisense strand	ACCACAUAGCGGUUUUUCC	50
si-TD093	Sense strand	AAACCGCUAUGUGGUGCUG	51
	Antisense strand	CAGCACCACAUAGCGGUUU	52
si-TD094	Sense strand	AACCGCUAUGUGGUGCUGA	53
	Antisense strand	UCAGCACCACAUAGCGGUU	54
si-TD096	Sense strand	CCGCUAUGUGGUGCUGAAA	55
	Antisense strand	UUUCAGCACCACAUAGCGG	56
si-TD097	Sense strand	CGCUAUGUGGUGCUGAAAG	57
	Antisense strand	CUUUCAGCACCACAUAGCG	58
si-TD098	Sense strand	GCUAUGUGGUGCUGAAAGG	59
	Antisense strand	CCUUUCAGCACCACAUAGC	60
si-TD136	Sense strand	GAGAAGGAGGUAAAAGAUG	61
	Antisense strand	CAUCUUUUACCUCCUUCUC	62
si-TD137	Sense strand	AGAAGGAGGUAAAAGAUGA	63
	Antisense strand	UCAUCUUUUACCUCCUUCU	64
si-TD138	Sense strand	GAAGGAGGUAAAAGAUGAG	65
	Antisense strand	CUCAUCUUUUACCUCCUUC	66
si-TD139	Sense strand	AAGGAGGUAAAAGAUGAGA	67
	Antisense strand	UCUCAUCUUUUACCUCCUU	68
si-TD140	Sense strand	AGGAGGUAAAAGAUGAGAA	69
	Antisense strand	UUCUCAUCUUUUACCUCCU	70

(continued)

siRNA	Sequence (5'-3')		SEQ ID NO
si-TD141	Sense strand	GGAGGUAAAAGAUGAGAAA	71
	Antisense strand	UUUCUCAUCUUUUACCUCC	72
si-TD143	Sense strand	AGGUAAAAGAUGAGAAAAA	73
	Antisense strand	UUUUUCUCAUCUUUUACCU	74
si-TD181	Sense strand	CUGAGUGACUAUGAGAAGU	75
	Antisense strand	ACUUCUCAUAGUCACUCAG	76
si-TD179	Sense strand	ACCUGAGUGACUAUGAGAA	77
	Antisense strand	UUCUCAUAGUCACUCAGGU	78
si-TD178	Sense strand	GACCUGAGUGACUAUGAGA	79
	Antisense strand	UCUCAUAGUCACUCAGGUC	80
si-TD177	Sense strand	UGACCUGAGUGACUAUGAG	81
	Antisense strand	CUCAUAGUCACUCAGGUCA	82
si-TD176	Sense strand	UUGACCUGAGUGACUAUGA	83
	Antisense strand	UCAUAGUCACUCAGGUCAA	84
si-TD224	Sense strand	GCAGGAGCAAGAAAAAUCA	85
	Antisense strand	UGAUUUUUCUUGCUCCUGC	86
si-TD221	Sense strand	AGAGCAGGAGCAAGAAAAA	87
	Antisense strand	UUUUUCUUGCUCCUGCUCU	88
si-TD217	Sense strand	UCCAAGAGCAGGAGCAAGA	89
	Antisense strand	UCUUGCUCCUGCUCUUGGA	90
si-TD380	Sense strand	UGAGGAGGACAGCUAUCUU	91
	Antisense strand	AAGAUAGCUGUCCUCCUCA	92
si-TD378	Sense strand	GUUGAGGAGGACAGCUAUC	93
	Antisense strand	GAUAGCUGUCCUCCUCAAC	94
si-TD376	Sense strand	CCGUUGAGGAGGACAGCUA	95
	Antisense strand	UAGCUGUCCUCCUCAACGG	96
si-TD372	Sense strand	GUCACCGUUGAGGAGGACA	97
	Antisense strand	UGUCCUCCUCAACGGUGAC	98
si-TD370	Sense strand	AGGUCACCGUUGAGGAGGA	99
	Antisense strand	UCCUCCUCAACGGUGACCU	100
si-TD364	Sense strand	UGGAUGAGGUCACCGUUGA	101
	Antisense strand	UCAACGGUGACCUCAUCCA	102
si-TD362	Sense strand	CUUGGAUGAGGUCACCGUU	103
	Antisense strand	AACGGUGACCUCAUCCAAG	104
si-TD358	Sense strand	GUAUCUUGGAUGAGGUCAC	105
	Antisense strand	GUGACCUCAUCCAAGAUAC	106
si-TD451	Sense strand	AGGAAGACCCUUCCCCUGA	107
	Antisense strand	UCAGGGGAAGGGUCUUCCU	108

(continued)

siRNA	Sequence (5'-3')		SEQ ID NO
si-TD443	Sense strand	GAUCCAAGAGGAAGACCCU	109
	Antisense strand	AGGGUCUUCCUCUUGGAUC	110
si-TD509	Sense strand	GGACAAGUCUGUGGCCCAG	111
	Antisense strand	CUGGGCCACAGACUUGUCC	112
si-TD508	Sense strand	UGGACAAGUCUGUGGCCCA	113
	Antisense strand	UGGGCCACAGACUUGUCCA	114
si-TD577	Sense strand	GCCUCCCUGGAGGAGAUCC	115
	Antisense strand	GGAUCUCCUCCAGGGAGGC	116
si-TD611	Sense strand	GGUAGCAAGGAAACUGGAG	117
	Antisense strand	CUCCAGUUUCCUUGCUACC	118
si-TD609	Sense strand	CUGGUAGCAAGGAAACUGG	119
	Antisense strand	CCAGUUUCCUUGCUACCAG	120
si-TD607	Sense strand	ACCUGGUAGCAAGGAAACU	121
	Antisense strand	AGUUUCCUUGCUACCAGGU	122
si-TD604	Sense strand	AGGACCUGGUAGCAAGGAA	123
	Antisense strand	UUCCUUGCUACCAGGUCCU	124
si-TD600	Sense strand	AUCCAGGACCUGGUAGCAA	125
	Antisense strand	UUGCUACCAGGUCCUGGAU	126
si-TD598	Sense strand	GGAUCCAGGACCUGGUAGC	127
	Antisense strand	GCUACCAGGUCCUGGAUCC	128
si-TD596	Sense strand	CCGGAUCCAGGACCUGGUA	129
	Antisense strand	UACCAGGUCCUGGAUCCGG	130
si-TD588	Sense strand	CAGCUGUCCCGGAUCCAGG	131
	Antisense strand	CCUGGAUCCGGGACAGCUG	132
si-TD587	Sense strand	GCAGCUGUCCCGGAUCCAG	133
	Antisense strand	CUGGAUCCGGGACAGCUGC	134
si-TD585	Sense strand	GGGCAGCUGUCCCGGAUCC	135
	Antisense strand	GGAUCCGGGACAGCUGCCC	136
si-TD720	Sense strand	GAGCUGAGAGACCUGUACA	137
	Antisense strand	UGUACAGGUCUCUCAGCUC	138
si-TD718	Sense strand	GGGAGCUGAGAGACCUGUA	139
	Antisense strand	UACAGGUCUCUCAGCUCCC	140
si-TD743	Sense strand	GACUCCCACCUCAGACAGA	141
	Antisense strand	UCUGUCUGAGGUGGGAGUC	142
si-TD145	Sense strand	GUAAAAGAUGAGAAAAAUA	143
	Antisense strand	UAUUUUUCUCAUCUUUUAC	144
si-TD480	Sense strand	UCUUGUGCUGAGAGCUUUC	145
	Antisense strand	GAAAGCUCUCAGCACAAGA	146

(continued)

siRNA	Sequence (5'-3')		SEQ ID NO
si-TD483	Sense strand	UGUGCUGAGAGCUUUCGGG	147
	Antisense strand	CCCGAAAGCUCUCAGCACA	148
si-TD736	Sense strand	ACAGACAGAUGGACCUGCA	149
	Antisense strand	UGCAGGUCCAUCUGUCUGU	150
si-TD734	Sense strand	GUACAGACAGAUGGACCUG	151
	Antisense strand	CAGGUCCAUCUGUCUGUAC	152
si-TD730	Sense strand	ACCUGUACAGACAGAUGGA	153
	Antisense strand	UCCAUCUGUCUGUACAGGU	154
si-TD726	Sense strand	AGAGACCUGUACAGACAGA	155
	Antisense strand	UCUGUCUGUACAGGUCUCU	156
si-TD723	Sense strand	CUGAGAGACCUGUACAGAC	157
	Antisense strand	GUCUGUACAGGUCUCUCAG	158
si-TD717	Sense strand	AGGGAGCUGAGAGACCUGU	159
	Antisense strand	ACAGGUCUCUCAGCUCCCU	160
si-7	Sense strand	UGGGAGAUGGGAAGCGAAA	161
	Antisense strand	UUUCGCUUCCCAUCUCCCA	162
si-10	Sense strand	CAGACAAAGGGGCCACCUA	163
	Antisense strand	UAGGUGGCCCCUUUGUCUG	164
si-1	Sense strand	GGACCUGGUAGCAAGGAAA	165
	Antisense strand	UUUCCUUGCUACCAGGUCC	166

III. Pharmaceutical Compositions

[0075] In yet another aspect, the present invention provides a pharmaceutical composition comprising at least one dsRNA of the present invention or expression vector comprising a nucleotide sequence encoding the dsRNA, and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical compositions are used for treating inflammatory diseases, such as arthritis, particularly rheumatoid arthritis (RA).

[0076] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal, intraarticular or epidermal administration (e.g., by injection or infusion). Depending on the route of administration, the active compound, i.e., the dsRNA molecule, can be encapsulated in a material, such as a liposome, to protect the compound from acids and other natural conditions that inactivate the compound. In some embodiments, the dsRNA of the present invention may be delivered by a cationic liposome delivery system.

[0077] The pharmaceutical compositions of the present invention may also contain pharmaceutically acceptable antioxidants. Examples of pharmaceutically acceptable antioxidants include: (1) Water-soluble antioxidants such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) Oil-soluble antioxidants such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, α-tocopherol and the like; and (3) metal chelating agents such as citric acid, ethylenediaminetetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid and the like.

[0078] Such compositions may also contain, for example, preservatives, wetting agents, emulsifying agents and dispersing agents.

[0079] Prevention of the presence of microorganisms can be ensured by sterilization procedures or by the inclusion of various antibacterial and antifungal agents such as parabens, chlorobutanol, phenol sorbic acid, and the like. In many cases, it is preferred to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium oxide in the composition. Prolonged absorption of the injectable pharmaceutical can be realized by adding to the composition of absorption delaying agents, for example, monostearate salts and gelatin.

[0080] Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The use of such media and agents for pharmaceutically active substances is well known in the art. Conventional media or agents, other than any range incompatible with the active compound, may be present in the pharmaceutical composition of the present invention. Additional active compounds may also be incorporated into the compositions.

[0081] Generally, therapeutic compositions must be sterile and stable under the conditions of manufacture and storage. The compositions may be formulated as solutions, microemulsions, liposomes or other ordered structures suitable for high drug concentrations. The carrier can be a solvent or dispersion containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

[0082] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in a suitable solvent with one or a combination of ingredients enumerated above, as required, followed by sterile microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile carrier which contains a basic dispersion medium and the other required ingredients from those enumerated above. For sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are drying under vacuum and freeze-drying (lyophilization) which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0083] The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form will vary depending upon the subject being treated and the particular mode of administration. The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Typically, this amount ranges from about 0.01% to about 99% active ingredient, e.g., from about 0.1% to about 70%, e.g., from about 1% to about 30% active ingredient, on a 100% basis, in combination with a pharmaceutically acceptable carrier.

[0084] The dosage regimen can be adjusted to provide the optimal desired response (e.g., therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased as required by the exigencies of the therapeutic situation. It is particularly advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit contains a predetermined amount of active compound calculated to produce the desired therapeutic effect in combination with the required pharmaceutical carrier. The specific description of the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art for formulating such active compounds for the treatment of sensitivity in individuals.

[0085] For administration of the dsRNA molecules of the present invention, the dosage may range from about 0.0000001 to 100 mg/kg body weight of the recipient. An exemplary treatment regimen may be once a week, once every two weeks, once every three weeks, once every four weeks, once a month, once every two months, once every three months, once every four months, once every five months, once every six months, once every seven months, once every eight months, once every nine months, once every 10 months, once every 11 months, even once every 12 months, or with a short administration interval at the beginning (such as once per week to once every three weeks), and then an extended interval later (such as once a month to even once every 12 months).

[0086] Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors, including the activity of the particular composition of the invention employed, way of administration, the time of administration, the rate of excretion of the particular compound employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular composition employed, the age, sex, weight, condition, general health, and medical history of the patient being treated, and similar factors known in the medical field.

IV. Disease Treatment

[0087] The present inventors designed more than 200 siRNA molecules directed against CKIP-1 gene, from which siRNA molecules capable of significantly inhibiting CKIP-1 expression were screened (Examples 1-3). Experiments show that the CKIP-1 targeting molecule of the present invention can significantly inhibit the expression of CKIP-1 protein in human osteoblasts (Example 5), and administration of the dsRNA can promote the expression of phenotypic genes of human osteoblasts, thereby promoting osteoblast differentiation (Examples 6 and 7). In mouse and cynomolgus monkey models, administration of dsRNA of the invention significantly alleviates arthritis progression (Examples 8 and 9).

[0088] Even more surprisingly, the present inventors have found that the dsRNA of the present invention is capable of inhibiting the expression of pro-inflammatory cytokines IL-6, TNF-α and/or IL-17A (Examples 4 and 8). In particular, the dsRNA of the present invention can significantly inhibit the expression of the pro-inflammatory cytokine IL-6. TNF-α is mainly expressed by macrophages of inflamed joints, synovial lining cells and activated T cells. In RA inflamed joints, TNF-α is one of the most prominent pro-inflammatory cytokines capable of inducing the production of other pro-inflammatory factors such as IL-1 β, IL-6 and IL-8. IL-6 receptor neutralizing antibodies completely abolish the inflammatory response during the induction of CIA, suggesting that IL-6 plays an important role in the initiation of arthritis.

[0089] While previous pharmaceutical studies on CKIP-1 have been focused primarily on inhibiting bone destruction or repairing bone damage, the present invention for the first time discovered that dsRNA targeting CKIP-1 of the present invention can inhibit the expression of pro-inflammatory cytokines, and thus can be used to treat inflammation. The dsRNA targeting CKIP-1 of the present invention capable of inhibiting inflammation is particularly advantageous in the treatment of arthritis, particularly rheumatoid arthritis, because in RA, the main early symptoms are joint inflammation, while bone destruction only occurs after several years (referred to as "late stage bone destruction"). The dsRNA targeting CKIP-1 of the present invention can inhibit inflammation and also repair bone damage, and thus can be advantageously used in various stages of RA treatment, without being limited to late stage bone destruction.

[0090] Accordingly, in another aspect, the present invention provides a method of treating arthritis, particularly rheumatoid arthritis, in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a dsRNA molecule of the present invention or an expression vector of the present invention or a pharmaceutical composition of the present invention.

[0091] In yet another aspect, the invention also provides the use of a dsRNA of the invention or an expression vector of the invention or a pharmaceutical composition of the invention in the preparation of a medicament for treating arthritis, particularly rheumatoid arthritis, in a subject in need thereof.

[0092] Arthritis that can be treated by the dsRNA molecules of the invention or expression vectors of the invention or pharmaceutical compositions of the invention include, but are not limited to, rheumatoid arthritis, osteoarthritis, idiopathic arthritis, ankylosing spondylitis, psoriatic arthritis, infectious arthritis, Juvenile arthritis, reactive arthritis, gouty arthritis, and the like.

[0093] The dsRNA of the invention or the expression vector of the invention or the pharmaceutical composition of the invention may also be used in combination with an additional therapeutic agent for the treatment of arthritis, in particular rheumatoid arthritis. Such additional therapeutic agents include, but are not limited to, non-steroidal anti-inflammatory drugs, hormones, anti-rheumatic drugs, and the like.

[0094] In yet another aspect, the invention provides a method of treating an inflammatory disease associated with a pro-inflammatory cytokine (e.g., IL-6, TNF-α and/or IL-17A) in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a dsRNA molecule of the invention or an expression vector of the invention or a pharmaceutical composition of the invention.

[0095] In yet another aspect, the invention also provides the use of a dsRNA of the invention or an expression vector of the invention or a pharmaceutical composition of the invention in the preparation of a medicament for the treatment of an inflammatory disease associated with a pro-inflammatory cytokine (e.g., IL-6, TNF-α and/or IL-17A) in a subject in need thereof.

[0096] Inflammatory diseases associated with such pro-inflammatory cytokines (e.g., IL-6, TNF-α, and/or IL-17A) include, but are not limited to, inflammatory bowel disease, inflammation caused by infection, inflammation caused by injury, inflammation of the respiratory system, inflammation associated with cancer, and the like. Inflammatory diseases associated with such pro-inflammatory cytokines (e.g., IL-6, TNF-α and/or IL-17A) also include arthritis, such as those listed above, particularly rheumatoid arthritis.

[0097] Other inflammatory diseases associated with pro-inflammatory cytokines (e.g., IL-6, TNF-α, and/or IL-17A) that can be treated by dsRNA molecules of the invention or expression vectors of the invention or pharmaceutical compositions of the invention include, but are not limited to, systemic lupus erythematosus, Crohn’s disease, psoriasis, colitis, ileitis, glomerulonephritis, asthma, dermatitis (including contact dermatitis and atopic dermatitis), vasculitis, chronic bronchitis, chronic prostatitis, appendicitis, pancreatitis, pelvic inflammation, polymyositis, chronic obstructive pulmonary disease and the like.

[0098] The dsRNA of the invention or the expression vector of the invention or the pharmaceutical composition of the invention may also be used in combination with additional therapeutic agents for the treatment of inflammatory diseases, in particular inflammatory diseases associated with pro-inflammatory cytokines such as IL-6, TNF-α and/or IL-17A. Such additional therapeutic agents are, for example, inhibitors that target TNF-α, including but not limited to Infliximab, Etanercept, Adalimumab, Golimumab, and Certolizumab; IL-6 blockers, including but not limited to, Tocilizumab; IL-17A blockers, including but not limited to Secukinumab.

[0099] In yet another aspect, the invention provides a method of treating a bone metabolism-related disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a dsRNA molecule of the invention or an expression vector of the invention or a pharmaceutical composition of the invention.

[0100] In yet another aspect, the invention also provides the use of a dsRNA of the invention or an expression vector of the invention or a pharmaceutical composition of the invention in the preparation of a medicament for a bone metabolism-related disorder in a subject in need thereof.

[0101] Such bone metabolism related diseases include, but are not limited to, osteomalacia, bone deficiency, osteolytic bone disease, renal bone disease, osteogenesis imperfecta, bone destruction caused by cancer bone metastases, and the like.

[0102] The dsRNA of the present invention or the expression vector of the present invention or the pharmaceutical composition of the present invention may also be used in combination with additional therapeutic agents for the treatment of bone metabolism-related disorder.

[0103] In yet another aspect, the invention provides a method of reducing the level of a pro-inflammatory cytokine (e.g., IL-6, TNF-α and/or IL-17A) in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a dsRNA molecule of the invention or an expression vector of the invention or a pharmaceutical composition of the invention.

[0104] Preferably, in the above aspects of the invention, the subject is human.

[0105] In some embodiments, the dsRNA of the invention or the expression vector of the invention or the pharmaceutical composition of the invention is administered intra-articularly. In some embodiments, the dsRNA of the invention or the expression vector of the invention or the pharmaceutical composition of the invention is administered systemically.

EXAMPLES

[0106] A further understanding of the present invention may be obtained by reference to specific examples set forth herein which are presented for purposes of illustration only and are not intended to limit the scope of the invention in any way. It will be evident that various modifications and changes may be made thereto without departing from the spirit of the invention, and accordingly, such modifications and changes are intended to be within the scope of the appended claims.

Example 1. Sequence Design and Synthesis of siRNA Targeting CKIP-1

[0107] Candidate siRNAs were designed according to homologous regions of human CKIP-1 mRNA and monkey CKIP-1 mRNA sequences to obtain a candidate siRNA sequence pool. Off-target effects were comprehensively analyzed for the candidate siRNA sequence pool, and candidate siRNA sequences with high off-target scores were removed. By combining a seed region matching score, 208 siRNA candidate sequences against CKIP-1were finally obtained and synthesized. Eight unrelated NC sequences were also designed and synthesized as negative controls in the screening assay. The synthesized 208 gene siRNA sequences, as well as 8 NC sequences, are as shown in Fig. 1.

Example 2. Real-time Quantitative PCR Screening for SiRNAs that Inhibit CIKP-1 Expression

[0108] HFOB cells (human osteoblast strain commercially available from the Chinese Academy of Sciences) were seeded in 96-well cell culture plates, and siRNA transfection was performed at a cell density of about 70%. 0.5 μl of Lipofectamine2000 was diluted in 25 μl of opti-MEM without serum and antibiotics and mixed well. 15 pmol of RNA was diluted in 25 μl of opti-MEM without serum and antibiotics and mixed slightly. The Lipofectamine2000 dilution was added to the RNA dilution and mixed well. It was left at room temperature for 20 min. A mixture of 50 μl Lipofectamine2000 and RNA was added to a 96-well cell plate seeded with cells, slightly shaken to mix well, and the solution was changed after 5 h. RNA was extracted 48 hours later (TIANGEN micro RNA extraction kit), and qPCR detection (TransGen qPCR kit) was performed after reverse transcription (Takara reverse transcription kit). The relative expression of CIKP-1 was determined using GADPH gene as internal reference. The corresponding CIKP-1 relative expression values of the obtained siRNAs were normalized to the blank treatment group. Each NC sequence was also used as a negative control. The primer sequence is as follows:

CIKP1-F: gGAACCAACCTCTTGTGCTG
CIKP1-R: gTCAACTTCTTGGGTGCCTG
GADPH-F: cATGAGAAGTATGACAACAGCCT
GADPH-R: aGTCCTTCCACGATACCAAAGT

[0109] The results showed that 82 sequences with an interference efficiency of 50% and above were selected from 208 siRNA sequences (see Table 2), and 22 sequences have an interference efficiency of 70% and above (shown in bold italics in Table 2). These sequences were used as candidates for further screening.

Table 2 siRNA with interference efficiency of 50%and above

SiRNA Sequence	**Normalized target gene relative expression value**
si-TD037	0.472339
si-TD040	0.457801
si-TD042	0.422001
si-TD044	0.398672
si-TD050	0.307432
si-TD057	0.417976
si-TD058	0.412397
si-TD060	***0.250549***
si-TD061	0.314191
si-TD062	***0.198302***
si-TD064	0.46957
si-TD065	0.4389
si-TD066	***0.30317***
si-TD067	0.411764
si-TD068	***0.252114***
si-TD070	***0.281898***
si-TD072	0.401834
si-TD074	***0.220171***
si-TD076	0.334746
si-TD078	0.318811
si-TD080	***0.23612***
si-TD082	***0.297076***
si-TD084	0.356374
si-TD087	0.32098
si-TD089	***0.238577***
si-TD093	0.367916
si-TD094	0.410962
si-TD096	***0.17883***
si-TD097	0.409968
si-TD098	0.431926
si-TD136	0.356366
si-TD137	***0.118522***
si-TD138	0.387089
si-TD139	0.335127
si-TD140	***0.235433***
si-TD141	***0.287318***
si-TD143	***0.169164***

(continued)

SiRNA Sequence	Normalized target gene relative expression value
si-TD145	0.346415
si-TD176	0.223003
si-TD177	0.410735
si-TD178	***0.172067***
si-TD179	0.469953
si-TD181	***0.224999***
si-TD217	0.414913
si-TD221	0.462056
si-TD224	0.490381
si-TD358	0.387057
si-TD362	***0.288363***
si-TD364	***0.275357***
si-TD370	0.445778
si-TD372	0.459658
si-TD376	0.387624
si-TD378	***0.295441***
si-TD380	0.400417
si-TD443	0.396858
si-TD451	0.311861
si-TD480	0.460598
si-TD483	0.377209
si-TD508	0.476182
si-TD509	0.468754
si-TD577	0.424962
si-TD585	0.448536
si-TD587	0.410307
si-TD588	0.441516
si-TD596	0.497351
si-TD598	0.422082
si-TD600	0.487359
si-TD604	0.401307
si-TD607	0.375209
si-TD609	0.476541
si-TD611	0.457187
si-TD717	0.467227
si-TD718	0.450869
si-TD720	0.335688
si-TD723	0.411798

(continued)

SiRNA Sequence	Normalized target gene relative expression value
si-TD726	***0.270674***
si-TD730	***0.252773***
si-TD734	0.48745
si-TD736	0.416718
si-TD743	0.471836
si-1	0.506191
si-7	0.373945

Example 3. Identification of Candidate siRNA by Dual Luciferase Assay

[0110] In this example, candidate siRNA sequences obtained in Example 2 were further identified by a dual luciferase assay.

1. Constructing target gene CKIP-1 overexpression vector

[0111] The sequence fragment of CKIP-1 CDS 1-652 was amplified by PCR using upstream and downstream primers with SacI and XhoI restriction enzyme cutting sites and protective bases, respectively. The amplification product was digested by SacI and XhoI and then inserted into a pGP-miRGLO overexpression vector (see Fig. 2) which was also digested by SacI and XhoI to obtain the pmirGlo-CDS1 carrier overexpressing the sequence of the first segment (1-652) of the CDS region of CKIP-1 gene.

[0112] The sequence fragment of CKIP-1 CDS 653-1230 was amplified by PCR using upstream and downstream primers with SacI and XhoI restriction enzyme cutting sites and protective bases, respectively. The amplification product was digested by SacI and XhoI and then inserted into a pGP-miRGLO overexpression vector (see Fig. 2) which was also digested by SacI and XhoI to obtain the pmirGlo-CDS2 carrier overexpressing the sequence of the second segment (653-1230) of the CDS region of CKIP-1 gene.

2. Cell culture

[0113] 293T cells were regularly cultured in DMEM medium (Gibco) containing 10% FBS (Gibco) (containing 1.5 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin) in an incubator with 5% CO2 saturated humidity under 37 °C.

3. Transfection of cells

[0114] When 293T cells were cultured until 80-90% confluence in a 10 cm dish, the medium was decanted, and the cells were washed twice with 3 ml PBS. 1 ml of Trypsin-EDTA solution was added, mixed well, carefully aspirated the pancreatin solution and placed at 37 °C for 2-3 minutes. 2 ml complete medium was added and the cells were pippeted to form a single cell suspension. The cells were counted and seeded in 24-well plates at approximately 1 x 10^5 cells per well.

[0115] 150 μl of Opti-MEMI (50 μl/well*3) was added to a 1.5 ml EP tube, followed by adding 30 ng of the corresponding plasmid (10 ng per well) and the corresponding amount of siRNA (each siRNA final concentration setting gradient: 6.25, 12.5, 25, 50, 100, 300 nM, the final concentration of the negative control NC-7 is 25 nM), and mixing well; another 1.5 ml EP tube was filled with 150 μl of Opti-MEMI (50 μl/well*3) and 6 μl of transfection reagent Lipo2000, mixed well, left standstill for 5 min and then mixed well in a volume of 300 ul for 20 min at room temperature. The culture medium was removed from the 24-well plate prepared on the previous day, and 400 μl/well culture medium was added; after being left standstill for 20 min, the transfection mixture was added to the above 24-well plate by 100 μl/well, with each 3 replicates, Blank wells and Mock wells were set, the plates were shaken well and incubated in an incubator for 6 hours. The transfection liquid was removed, rinsed with PBS, culture medium was added in for continuous culture, and photos of the wells transfected with NC-FAM were taken to observe the transfection efficiency. Cells were collected 24 h after transfection for dual luciferase assay.

4. Dual luciferase assay

[0116] Experimental materials and reagents: Dual-Luciferase Reporter Assay System(Promega, E1960); PBS; 96-well white plate (corning cat.#3912); Multilabel Microplate Detector (PerkinElmer EnSpire).

[0117] Experimental steps: the medium was removed from the cell plates to be examined, the cultured cells were washed with PBS, and the PBS was aspirated and discarded. 1 x PLB was added by 100 μl/well, and the plates were slightly shaken at room temperature and lysed for 15 min. The cell lysate was moved into a small centrifuge tube for centrifugation at 3000rpm for 3min, cell debris were removed, 30 μl of supernatant was taken, added to a 96-well white plate, and a substrate was added for detection according to the recommended operation steps of the specification.

Results:

[0118] Four siRNAs have good inhibition effects on CKIP-1 expression in a dual luciferase assay: si-TD137, si-TD141, si-TD176, and si-7. The results are shown in Tables 3-6 and Figs. 3-6, respectively.

Table 3 Dual luciferase assay results for si-TD137

	Total mean value of normalized dual luciferase relative activity	**Standard deviation**	**P-value compared with NC7 group**
empty vector	261.5710548	96.3502	
empty vector+NC-7	252.5532524	80.68784	
empty vector+137-6.25	222.8452964	63.83427	
empty vector+137-12.5	228.8008311	67.97434	
empty vector+137-25	224.1443386	70.3993	
empty vector+137-50	233.7895134	96.01054	
empty vector+137-100	240.7744442	70.31852	
empty vector+137-300	224.4435964	65.32518	
CDS1	100	0	
CDS1+NC-7	97.47065705	27.59265	
CDS1+137-6.25	12.30603204	2.139099	0.005965
CDS1+137-12.5	10.86442887	2.108049	0.005615
CDS1+137-25	10.28528737	1.054173	0.005438
CDS1+137-50	9.281597349	2.315117	0.005271
CDS1+137-100	10.82386854	1.645889	0.005582
CDS1+137-300	7.593906894	3.041176	0.004967

Table 4 Dual luciferase assay results for si-TD141

	Total mean value of normalized dual luciferase relative activity	**Standard deviation**	**P-value compared with NC7 group**
empty vector	232.9723364	61.66293	
empty vector+NC-7	205.4147266	20.38412	
empty vector+141-6.25	215.5570016	37.7184	
empty vector+141-12.5	210.1388575	23.7144	
empty vector+141-25	217.6250075	31.30451	
empty vector+141-50	195.7261375	45.5828	
empty vector+141-100	225.8556173	53.21314	

(continued)

	Total mean value of normalized dual luciferase relative activity	**Standard deviation**	**P-value compared with NC7 group**
empty vector+141-300	194.396959	47.47294	
CDS1	100	0	
CDS1+NC-7	95.13464683	19.17207	
CDS1+141-6.25	16.98508418	2.229641	0.002177
CDS1+141-12.5	14.2576769	3.90799	0.002015
CDS1+141-25	12.61787894	3.959899	0.001872
CDS1+141-50	12.64749866	2.563133	0.001791
CDS1+141-100	12.05755431	0.886328	0.001693
CDS1+141-300	8.134042105	2.163326	0.00145

Table 5 Dual luciferase assay results for si-TD176

	Total mean value of normalized dual luciferase relative activity	**Standard deviation**	**P-value compared with NC7 group**
empty vector	259.3844179	14.91878	
empty vector+NC-7	253.6837275	24.80359	
empty vector+176-6.25	232.3986339	17.01937	
empty vector+176-12.5	199.7533446	2.950685	
empty vector+176-25	218.9236568	32.63606	
empty vector+176-50	203.8520998	31.30066	
empty vector+176-100	219.0134189	30.88615	
empty vector+176-300	186.6665828	16.72755	
CDS1	100	0	
CDS1+NC-7	94.53375651	4.874274	
CDS1+176-6.25	21.31523373	1.321383	1.49E-05
CDS1+176-12.5	21.95335577	2.282599	1.99E-05
CDS1+176-25	20.89875168	1.167482	1.42E-05
CDS1+176-50	19.27982377	0.247705	1.17E-05
CDS1+176-100	21.66118398	3.755765	3.34E-05
CDS1+176-300	18.16630661	1.603929	1.35E-05

Table 6 Dual luciferase assay results for si-7

	Total mean value of normalized dual luciferase relative activity	**Standard deviation**	**P-value compared with NC7 group**
empty vector	161.0586219	66.14465	
empty vector+NC-7	164.50757	62.34558	
empty vector+si7-6.25	169.6545487	57.58843	
empty vector+si7-12.5	170.6842599	53.23935	

(continued)

	Total mean value of normalized dual luciferase relative activity	**Standard deviation**	**P-value compared with NC7 group**
empty vector+si7-25	162.2483155	54.75665	
empty vector+si7-50	171.4671143	51.29689	
empty vector+si7-100	172.0681778	62.19204	
empty vector+si7-300	163.0764404	51.25441	
CDS2	100	0	
CDS2+NC-7	99.61211154	15.69399	
CDS2+si7-6.25	17.29806814	2.382198	0.00085
CDS2+si7-12.5	18.13340456	1.80696	0.000868
CDS2+si7-25	17.09295528	1.84478	0.000828
CDS2+si7-50	18.07601932	0.777867	0.000848
CDS2+si7-100	17.5441839	3.941363	0.000926
CDS2+si7-300	15.54928456	3.365782	0.000819

Example 4. siRNA targeting CKIP-1 inhibits expression of pro-inflammatory cytokine

[0119] RAW264.7 mouse peritoneal macrophage cell line (purchased from Cell Bank of Chinese Academy of Sciences, Shanghai) was cultured in complete DMEM medium containing 10% fetal bovine serum, 100 U penicillin and streptomycin, and cultured overnight in an incubator under 37 °C constant temperature carbon dioxide (5%) until cell confluence reached 70-80%.

[0120] In vitro, mouse macrophages transfected with the small interfering RNAs against CKIP-1 prepared as described above or their modified forms having methoxy group modification on sense strand were used as a drug treatment group (RNAi group), and the cells treated with the transfection reagent X-TremeGENE siRNA transfection reagent alone (commercially available from Roche, article number 4476093001) were used as a transfection reagent group (MOCK group), 3 in each group in parallel, at least three times for each experiment. For transfection of mouse macrophages, the final concentration of small interfering RNA was 30 nM. 24 hours after transfection, LPS (commercially available from Sigma, Cat. No. L2630-10MG) was added to stimulate for 6 hours, supernatants of cells of each group were collected, secretion of proinflammatory cytokines was detected, and mRNA expression levels of proinflammatory cytokines were detected by collecting cells of each group.

1. Determination of TNF-α and IL-6 protein secretion inhibition efficiency by siRNA

[0121] Inhibition efficiency of the secretion levels of TNF-α and IL-6 in the cell supernatant was determined by an ELISA method, specifically: Mouse TNF alpha ELISA Ready-SET-Go!®(eBioscience, Cat. No. 88-7324-88) and Mouse IL-6 ELISA Ready-SET-Go!(eBioscience, Cat. No. 88-7064-88) kits were used according to the instructions, and the concentrations of TNF-α and IL-6 were calculated by plotting standard curves.

[0122] Cytokine inhibition efficiency was calculated as follows:

Cytokine inhibition efficiency = [(LPS group-treated group)/(LPS group-blank control group)] x 100%.

[0123] The results of the determination are shown in Tables 7 and 8 below:

Table 7

		Inhibition (%) of TNFα secretion	Inhibition of IL-6 secretion (%)
MOCK group	Transfection reagent	3.71	29.81

(continued)

		Inhibition (%) of TNFα secretion	Inhibition of IL-6 secretion (%)
RNAi group	si-7	32.23	77.13*
	si-137	25.95	55.58*
	si-141	21.49	65.30*
	si-176	17.75	56.69*
Note:*P <0.05, compared with MOCK group, there was statistically significant difference.			

Table 8

		Inhibition (%) of TNF α secretion	Inhibition of IL-6 secretion (%)
MOCK group	Transfection reagent	1.28	21.62
RNAi group	si-7-Ome	35.51	89.75*
	si-137-Ome	26.30	84.51*
	si-141-Ome	27.95	87.85*
	si-176-Ome	21.10	80.12*
Notes: *P <0.05, compared with MOCK group, there was statistically significant difference.			

[0124] As can be seen from the above table, each candidate siRNA and its methylation modified form inhibited LPS-induced IL-6 and TNF-α secretion from RAW264.7 mouse macrophages, and the inhibition of IL-6 secretion reached a significant level.

2. Determination of TNF-α and IL-6 mRNA expression inhibition efficiency by siRNA

[0125] TNF-α and IL-6 mRNA levels in the collected RAW264.7 cells were detected by a real-time fluorescence quantitative PCR (real-time PCR) method, specifically: total cellular RNA was extracted using TRIzol reagent (Invitrogen, article number 15596018), cDNA was synthesized by reverse transcription using TransScriptAll-in-One First-Strand cDNA Synthesis SuperMixforq PCR (One-Step DNA Removal) (TransGen, article number AT341-02) kit, and the inhibitory efficiency of siRNA on LPS-induced IL-6 and TNF-α expression in murine peritoneal macrophages was detected by fluorescence quantitative PCR.

[0126] The GAPDH gene was used as an internal reference gene in the Real-time PCR method, and the primer sequences were shown in Table 9

Table 9

	Forward (5'-3')	Reverse (5'-3')
Mice TNF-α	TCAGCGAGGACAGCAAGG	AGTGAGTGAAAGGGACAGAACC
Mice IL-6	CCTTCTTGGGACTGATGCTG	TTGGGAGTGGTATCCTCTGTGA
Mice GAPDH	CCTTCATTGACCTCAACTACATGG	CTCGCTCCTGGAAGATGGTG

[0127] In the fluorescence quantitative PCR method, the nucleic acid inhibition efficiency is calculated according to the following equation:
siRNA inhibition efficiency = [(LPS group cytokine gene copy number/LPS group GAPDH gene copy number-treatment group cytokine gene copy number/treatment group GAPDH gene copy number)/(LPS group cytokine gene copy number/LPS group GAPDH gene copy number-blank control group cytokine gene copy number/blank control group GAPDH gene copy number)] x 100%

[0128] The results are shown in Table 10:

Table 10

		Inhibition (%) of TNFα secretion	Inhibition of IL-6 secretion (%)
MOCK group	Transfection reagent	11.13	11.96
RNAi group	si-7	-4.6	70.75*
	si-137	6.64	40.78*
	si-141	-1.58	54.53*
	si-176	6.80	45.76*
Notes: *P <0.05, compared with MOCK group, there was statistically significant difference.			

[0129] As can be seen from Table 10, each candidate siRNA significantly inhibited LPS-induced IL-6 mRNA expression in mouse macrophages; there was no significant inhibitory effect on TNF-α mRNA expression.

[0130] It can be seen that the CKIP-1 targeting siRNAs of the present invention can inhibit the levels of the pro-inflammatory cytokines IL-6 and TNF-α, particularly IL-6, thereby inhibiting inflammation, particularly inflammation associated with IL-6 and/or TNF-α, such as inflammation in RA.

Example 5. Inhibitory effect of siRNA targeting CKIP-1 on CKIP-1 protein expression

[0131] Human osteoblast cell line hFOB1.19 purchased from the Chinese Academy of Sciences cell bank was cultured in DMEM-F12 medium containing 10% fetal bovine serum (purchased from Gibco). Human osteoblast hFOB1.19 was transferred to 24-well plates for overnight culture for adhesion. Human osteoblast hFOB1.19 transfected with siRNA targeting CKIP-1 was used as treatment group, and the cells transfected with non-specific nucleic acid were used as negative control group (NC group). Each group with 2 duplicates was repeated at least 3 times. Human osteoblast cells were transfected with a final nucleic acid concentration of 20 μM. After 72 hours of transfection, cells were collected and assayed for CKIP-1 protein expression.

[0132] The content of CKIP-1 protein in osteoblast cells was detected by immunoblotting according to the method in the literature (Molecular Cloning A Laboratory Manual, Science Press, 2005). The CKIP-1 antibody used for immunoblotting was purchased from Santa Cruz Biotechnology (Cat. No. sc-376355) and the internal reference antibody was GADPH (purchased from Santa Cruz Biotechnology, Cat. No. sc-166574).

[0133] In immunoblotting, nucleic acid inhibitory activity was calculated as follows: nucleic acid inhibitory activity = [1-(light intensity value of CKIP-1 Western blot band of treatment group/light intensity value of GAPDH Western blot band of treatment group)/(light intensity value of CKIP-1 Western blot band of control group/light intensity value of GAPDH Western blot band of control group)] x 100%.

[0134] Result: the expression of CKIP-1 in hFOB1.19 cells was significantly inhibited by si-7. Compared with control NC, there was significant difference (P <0.05). The determined results are shown in Table 11.

Table 11

	Inhibition rate (%) of CKIP-1 protein expression
NC	0.0
si-7	74.5*

Example 6. Effect of siRNA targeting CKIP-1 on osteoblast differentiation

[0135] Similar to Example 5, CKIP-1 siRNA was tested for mRNA expression levels of human osteoblast hFOB1.19 phenotypic gene alkaline phosphatase (ALP), type I collagen (COL1), osteopontin (OPN), bone sialoprotein (BSP) and osteocalcin (OC) over time using primers as shown in Table 12. The determined results are shown in Table 13.

Table 12

Gene	Primer sequence (5'-3')		Product Size	Tm	Genbank No.
	Forward	Reverse			
HumanALP	GTCAGCTCCACC ACAACCCT	GCCCTCATTGGC CTTCACCC	155	60	NM_000478.3
HumanCOL1	CACTGGTGATGC TGGTCCTG	CGAGGTCACGG TCACGAAC	179	60	NM_000088.3
HumanOPN	GTACCCTGATGC TACAGACG	TTCATAACTGTC CTTCCCAC	139	60	NM_ 001040060.1
HumanBSP	GGCACCTCGAA GACAACAAC	GCCCGTGTATTC GTACTCCC	135	60	NM_004967.3
HumanOC	AGGGCAGCGAG GTAGTGAAG	TGTGGTCAGCC AACTCGTCA	138	60	NM_199173.3
HumanGAPDH	GGCATGGACTGT GGTCATGAG	TGCACCACCAA CTGCTTAGC	87	60	NM_002046.3

Table 13:

	Increasing rate of ALP mRNA (%)	Increasing rate of COL1 mRNA (%)	Increasing rate of OPN mRNA (%)	Increasing rate of BSP mRNA (%)	Increasing rate of OC mRNA (%)
NC	0	0	0	0	0
si-7	86.5*	70.2*	93.0*	171.6*	85.11*
*P<0.05: compared with NC group, there was statistical difference.					

[0136] Result: ALP, COL1A1 and OPN begin to express at the early stage of osteoblast differentiation, while BSP and OC begin to express at the mature stage of osteoblast. After 72 hours of action, the expression of ALP, COL1, OPN, BSP and OC in si-7 group were significantly higher than that in NC group.

[0137] The experimental results show that the siRNA targeting CKIP-1 can increase the expression of the phenotypic genes of the human osteoblast cell line hFOB1.19, thereby promoting osteoblast differentiation.

Example 7. Effect of siRNA targeting CKIP-1 on bone matrix mineralization deposition rate

[0138] Calcium deposition is a key functional mineralization marker for mature osteoblasts during osteoblast formation in vitro. As described above, the human osteoblast cell line hFOB1.19 transfected with siRNA targeting CKIP-1 was used as a treatment group, and the cells transfected with non-specific nucleic acid were used as a negative control group (NC group). The final nucleic acid concentration for transfection was 20 μM. The frequency of interval transfection was once a week, with 4 duplicates per group in parallel. Calcium deposition in human osteoblast cell line hFOB1.19 was determined by calcium staining 7, 14 and 21 days after the first transfection.

[0139] The results are shown in Table 14. 21 days after the first transfection of the human osteoblast cells, the calcium deposition of the treatment group is obviously higher than that of the NC group, which at the functional level verifies that the siRNA can promote the differentiation of human preosteoblasts into mature osteoblasts.

Table 14:

		Calcium deposition in human osteoblasts (ng/μg protein)
NC Group	Non-specific nucleic acid	0.00

(continued)

		Calcium deposition in human osteoblasts (ng/μg protein)
Treatment group	si-7	64.81*
*P<0.05: compared with NC group, there was statistical difference.		

Example 8. Assessment of siRNA activity in vivo using a mice CIA model

[0140] Collagen-induced arthritis (CIA) model was established in 8-10 week old male DBA mice by subcutaneous injection of type II collagen at the root of the tail. The specific method is as follows: a proper amount of bovine type II collagen with a concentration of 2mg/mL was mixed with an equivalent amount of incomplete Freund's adjuvant, fully emulsified, and the emulsified mixture was injected subcutaneously at the root of the tail with 100 μg of type II collagen/mouse. After 21 days, 50 μg of type II collagen was injected subcutaneously once at the root of the tail for boost.

[0141] 5-grade semi-quantitative evaluation standard was taken as the evaluation standard of arthritis clinical severity: 0: no red swelling; 1: erythema accompanied with moderate swelling and confined to the midfoot or ankle; 2: moderate swelling extended from the ankle to the midfoot; 3: moderate swelling extended from the ankle joint to the metatarsal joint; 4: severe swelling of ankle, foot and toes.

[0142] The animals were randomly grouped if the severity of the double hindlimbs of each group of animals was scored about 1 through visual evaluation: carrier group, ankle joint cavity was injected with blank liposome; NC (negative control) group, liposomes loaded with negative control sequences were injected into the ankle joint cavity; the treatment group, liposomes loaded with Si-7, Si-137, Si-141 or Si-176 were injected into ankle joint cavity; the positive control group was administered with the positive drug Etanercept (purchased from Shanghai CP Guojian Pharmaceutical Co., Ltd., each containing 12.5 mg of active ingredient).

[0143] The animals in each group were administered by injecting into the bilateral ankle joint cavities of the hind limbs at a dose of 4 μg siRNA/5 μl liposome/ankle joint, six times on day 0, day 7, day 14, day 21, day 28 and day 35. The positive drug was administered by subcutaneous injection at a dose of 7.5 mg/kg body weight.

1. Effect of siRNA treatment on clinical score and body weight of CIA mice

[0144] The scores for swelling in the ankle joint of both hind limbs of mice were observed and recorded from the day of start twice a week, and the scores for the ankle joint of both hind limbs were added for statistical analysis. Clinical scoring results are shown in Fig. 7 and Table 15 below. Meanwhile, the body weight of the mice was recorded once a week, and the results are shown in FIG. 8 and Table 16.

[0145] The results showed that the body weight of each group of mice increased without weight loss. Si-7-Ome, Si-137-Ome, Si-141-Ome, and Si-176-Ome all significantly reduced the clinical score of mice CIA model arthritis, the inhibition rates were 50%, 60%, 70% and 60%, respectively, and the effects were better than the positive drug Etanercept (inhibition 40%).

Table 15. Inhibition of mice CIA clinical scores

Grouping		Mice CIA clinical score	Inhibition rate of mice CIA clinical score (%)
Carrier groups	Carrier	3.33	
NC group	Nonspecific nucleic acid	2.50	24.99
PC group	Etanercept	2.00	39.99
Treatment group	Si-7-Ome	1.67*	49.98
	Si-137-Ome	1.33**	60.01
	Si-141-Ome	1.00***	70.00
	Si-176-Ome	1.33**	60.01
Notes:*indicates P <0.05 as compared with the carrier groups, ** indicates P <0.01 as compared with the carrier groups, ** indicates P <0.001 as compared with the carrier groups.			

Table 16. Effect on body weight change in mice

		Day 0	Day 42	Weight change rate (%) of CIA mice
Carrier groups	Carrier	16.8	23.7	141.1
NC group	Nonspecific nucleic acid	17.4	24.3	139.7
PC group	Etanercept	16.6	23.0	138.6
Treatment group	Si-7-OMe	16.4	22.4	136.6
	Si-137-OMe	16.0	21.6	135.0
	Si-141-OMe	15.6	21.7	139.1
	Si-176-OMe	15.9	24.0	150.9

2. Effect of siRNA on expression of proinflammatory factors in joint tissue of CIA mice

[0146] After sacrifice, the fur of CIA model mice was cut off from the legs with scissors, so that the ankle joint was exposed, the portion below knee joint was cut off with forceps, cooled with liquid nitrogen, grinded and transferred to an enzyme-free tube, and total cellular RNA was extracted using TRIzol reagent (Invitrogen, article number 15596018). cDNA was synthesized by reverse transcription using TransScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal) (TransGen, article number AT341-02) kit. The inhibitory effects of si-7, si-137, si-141 and si-176 on CKIP-1, IL-6, TNF-α and IL-17A mRNA expression in joint tissue of CIA mouse model were detected by fluorescence quantitative PCR.

[0147] Primers for IL-6, TNF-α, and the reference gene GAPDH as described above were used. The CKIP-1, IL-17A primer sequences are shown in Table 17:

Table 17

	Forward (5'-3')	Reverse (5'-3')
Mice IL-17A	CTCCACCGCAATGAAGACC	CCCTCTTCAGGACCAGGATC
Mice CKIP-1	TTTCTCGGCCTTGGGAAAAC	GAGGCACATCGGCTCTTCT

[0148] In the fluorescent quantitative PCR method, the expression inhibition efficiency is calculated as follows:

Inhibition efficiency = [(cytokine gene copy number of the carrier groups/GAPDH gene copy number-treatment group cytokine gene copy number of the carrier groups/GAPDH gene copy number of the treatment groups)/(cytokine gene copy number of the carrier groups/GAPDH gene copy number-cytokine gene copy number of the normal control groups/GAPDH gene copy number of the normal control groups)] x 100%

[0149] The results of the determination are shown in Table 18 and Fig. 9:

Table 18

		Inhibition rate of CKIP-1 mRNA (%)	Inhibition rate of IL-6 mRNA (%)	Inhibition rate of TNF-α mRNA (%)	Inhibition rate of IL-17A mRNA (%)
Carrier groups	Empty liposome	0	0	0	0
NC group	Non-specific nucleic acid	7.10	-28.46	-16.43	29.42
Positive control group	Etanercept	41.37*	113.83***	127.36****	92.10***

(continued)

		Inhibition rate of CKIP-1 mRNA (%)	Inhibition rate of IL-6 mRNA (%)	Inhibition rate of TNF-α mRNA (%)	Inhibition rate of IL-17A mRNA (%)
Treatment group	Si-7-OMe	86.60****	83.17**	142.72****	126.59****
	Si-137-OMe	70.83****	135.84****	147.90****	154.57****
	Si-141-OMe	62.04***	68.85*	88.25****	118.81****
	Si-176-OMe	65.02***	68.85*	131.98****	65.53*
Notes:*P <0.05, compared with carrier group, there was statistically significant difference; ** P <0.01, compared with carrier group, there was statistically significant difference; *** P <0.001, compared with carrier group, there was statistically significant difference; **** P <0.0001, compared with carrier group, there was statistically significant difference; ### P <0.0001 compared with the normal control group, there was statistically significant difference.					

[0150] It can be seen that si-7-OMe, si-137-OMe, si-141-OMe, si-176-OMe all significantly inhibited the expression of CKIP-1, IL-6, TNF-α, IL-17AmRNA in the joint tissues of CIA mice, with inhibition rates more than 50%, and the inhibition of CKIP-1 mRNA was stronger than that of the positive drug Etanercept (41.37%). Compared with other small interfering RNAs, si-137 showed a stronger inhibitory effect on pro-inflammatory factors IL-6, TNF-α, IL-17AmRNA, and the inhibitory effect was stronger than that of the positive drug Etanercept. The siRNAs of the present invention are shown to be effective in inhibiting inflammation in RA.

3. Micro CT detection

[0151] Scancoviva CT 40 was used for the micro-CT detection. A mouse hind paw was put into a Micro CT sample tube for three-dimensional CT scanning and reconstruction. After the scanning, a matched software was used for analyzing the three-dimensional microstructure of the trabeculae and collecting the spatial structure parameters of the trabeculae.

4. Pathological examination

[0152] The hind limbs of mice were fixed in 4% formaldehyde solution and embedded with paraffin after EDTA decalcification. The pathological changes of joints and bone erosion were examined through serial section and HE staining.

5. Bone morphometric analysis

[0153] Mice were intraperitoneally injected with xylenol orange (90 mg/kg) 12 days before sacrifice and intraperitoneally injected with calcein (10 mg/kg) 2 days before sacrifice. After sacrifice, hind paws were removed and 10 μm discrete sections were made using a non-decalcifying microtome. Sections were stained with 1% methylene blue followed by light microscopy and unstained sections were used for fluorescence microscopy. The metatarsal bones in the paws were used for bone morphometric analysis.

[0154] Compared with the control group, each siRNA administration group played an positive role in improving inflammation and bone injury of a rheumatoid arthritis model and delaying disease progress, exhibiting good therapeutic effect.

Example 9. Validation of Effect of the siRNAs with Monkey Rheumatoid Arthritis Model

1. Animal modeling and administration

[0155] 3-6-year-old female cynomolgus monkeys were immunized with bovine type II collagen on day 0 and day 21, respectively, according to modeling methods of collagen-induced arthritis described in related literatures. The drug is locally administered into the joint after the onset of the disease. Liposome delivery systems were used for the small nucleic acids.

[0156] Grouping is as follows: as for the carrier groups, empty liposomes were injected into the joint cavity; as for NC (negative control) groups, liposomes loaded with negative control sequences were injected into ankle joint cavity; as for the treatment group, liposomes loaded with Si-7, Si-137, Si-141 or Si-176 were injected into the articular cavity; as for the positive control group, the positive drug etanercept (purchased from Shanghai CP Guojian Pharmaceutical Co., Ltd.)

was administered. Three animals in each group were administered by articular injection once a week for 6 weeks.

2. Detection of parameters

[0157] MicroCT, pathology, bone morphometry and other tests were similar to experiments in mice.

[0158] Compared with the control group, the siRNA administration group showed good treatment effects on improving disease condition, especially reducing bone damage, maintaining bone function and the like in a rheumatoid arthritis model.

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cuuggaugag gucaccguu                                                  19


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<400>  135
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<210>  157
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<400>  158
gucuguacag gucucucag                                                    19


<210>  159
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<400>  165
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ggaaccaacc tcttgtgctg                                                  20
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<220>
<223>  hGADPH-F

<400>  169
catgagaagt atgacaacag cct                                                23


<210>  170
<211>  22
<212>  DNA
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<220>
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<400>  170
agtccttcca cgataccaaa gt                                                 22


<210>  171
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<220>
<223>  mouse TNF-a-F

<400>  171
tcagcgagga cagcaagg                                                      18


<210>  172
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<220>
<223>  mouse TNFa-R

<400>  172
agtgagtgaa agggacagaa cc                                                 22


<210>  173
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<220>
<223>  mouse IL6-F

<400>  173
ccttcttggg actgatgctg                                                   20


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<400>  174
ttgggagtgg tatcctctgt ga                                                22


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<220>
<223>  mouse GAPDH-F

<400>  175
ccttcattga cctcaactac atgg                                              24


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<220>
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ctcgctcctg gaagatggtg                                                   20


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<210>  178
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<210>  182
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ttcataactg tccttcccac                                                    20


<210>  183
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<400>  183
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ggcacctcga agacaacaac                                                    20


<210>  184
<211>  20
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<220>
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<400>  184
gcccgtgtat tcgtactccc                                                    20


<210>  185
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<223>  OC-F

<400>  185
agggcagcga ggtagtgaag                                                    20


<210>  186
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<400>  186
tgtggtcagc caactcgtca                                                    20


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<220>
<223>  GAPDH-F

<400>  187
ggcatggact gtggtcatga g                                                  21


<210>  188
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<220>
<223>  GAPDH-R

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tgcaccacca actgcttagc                                                    20
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<220>
<223>  mouse IL-17A-F

<400>  189
ctccaccgca atgaagacc                                                    19


<210>  190
<211>  20
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<220>
<223>  mouse IL-17A-R

<400>  190
ccctcttcag gaccaggatc                                                   20


<210>  191
<211>  21
<212>  DNA
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<220>
<223>  mouse CKIP-1-F

<400>  191
tttctcggcc ttgggaaaaa c                                                 21


<210>  192
<211>  19
<212>  DNA
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<220>
<223>  mouse CKIP-1-R

<400>  192
gaggcacatc ggctcttct                                                    19
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Claims

1. A double-stranded RNA (dsRNA) molecule comprising a sense strand and an antisense strand which are selected from the group consisting of:

   1) a sense strand shown in SEQ ID NO: 63 and an antisense strand shown in SEQ ID NO: 64;
   2) a sense strand shown in SEQ ID NO: 71 and an antisense strand shown in SEQ ID NO: 72;
   3) a sense strand shown in SEQ ID NO: 83 and an antisense strand shown in SEQ ID NO: 84; and
   4) a sense strand shown in SEQ ID NO: 161 and an antisense strand shown in SEQ ID NO: 162.

2. The dsRNA molecule of claim 1, wherein the sense and/or antisense strand additionally has an overhang of at least one nucleotide at the 3' end.

3. The dsRNA molecule of claim 2, wherein the sense and/or antisense strand additionally has an overhang of 2 nucleotides at the 3' end, preferably the overhang is TT.

4. The dsRNA molecule of claim 1, wherein the sense strand and the antisense strand comprise 1 or 2 nucleotide substitutions located within 6, 5, 4, 3 or 2 nucleotides from the 5' and/or 3' end.

5. The dsRNA molecule of claim 4, wherein the sense and antisense strands comprise 1 nucleotide substitution, which is located at the last nucleotide of the 3' end of the sense strand and correspondingly at the first nucleotide of the 5' end of the antisense strand.

6. The dsRNA molecule of any one of claims 1-5, comprising at least one modified nucleotide.

7. The dsRNA molecule of claim 6, wherein the modified nucleotide is selected from the group consisting of: 2'-O-methyl modified nucleotides, 2'-F modified nucleotides, nucleotides containing 5'-phosphorothioate groups and end nucleotides linked to cholesteryl derivatives or dodecanoic acid bisdecylamide groups, 2'-deoxy-2'-fluoro modified nucleotides, 2'-deoxy-modified nucleotides, locked nucleotides, abasic nucleotides, 2'-amino-modified nucleotides, 2'-alkyl-modified nucleotides, morpholino nucleotides, phosphoramidates and nucleotides containing non-natural bases.

8. The dsRNA molecule of claim 6, wherein the 2' hydroxyl groups of all nucleotides with uracil or cytosine bases in the sense and/or antisense strands are modified with methoxy groups.

9. The dsRNA molecule of any one of claims 1-8, wherein it is an siRNA or shRNA.

10. The dsRNA molecule of any of claims 1-9, wherein it inhibits CKIP-1 expression by at least 50%, preferably by at least 70%.

11. The dsRNA molecule of any of claims 1-10, wherein it inhibits the expression of pro-inflammatory cytokine such as TNF-α, IL-6 and/or IL-17A.

12. An expression vector comprising a nucleotide sequence encoding the dsRNA molecule of any one of claims 1-11, wherein the nucleotide sequence is operably linked to a transcriptional regulatory element.

13. A pharmaceutical composition comprising the dsRNA molecule of any one of claims 1-11 or the expression vector of claim 12, and a pharmaceutically acceptable carrier.

14. A method for treating arthritis in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the dsRNA molecule of any one of claims 1-11 or the expression vector of claim 12 or the pharmaceutical composition of claim 13.

15. The method of claim 14, further comprising administering to the subject an additional therapeutic agent for treating arthritis.

16. Use of the dsRNA molecule of any one of claims 1-11 or the expression vector of claim 12 or the pharmaceutical composition of claim 13 in the preparation of a medicament for treating arthritis in a subject in need thereof.

17. The method of claim 14 or 15, or the use of claim 16, wherein the arthritis is selected from the group consisting of rheumatoid arthritis, osteoarthritis, idiopathic arthritis, ankylosing spondylitis, psoriatic arthritis, infectious arthritis, juvenile arthritis, reactive arthritis, gouty arthritis, preferably rheumatoid arthritis.

18. A method of treating an inflammatory disease, in particular a pro-inflammatory cytokine such as TNF-α, IL-6 and/or IL-17A related inflammatory disease, in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a dsRNA molecule according to any one of claims 1 to 11 or an expression vector according to claim 12 or a pharmaceutical composition according to claim 13.

19. The method of claim 18, further comprising administering to the subject an additional therapeutic agent for treating an inflammatory disease, particularly a pro-inflammatory cytokine such as TNF-α, IL-6 and/or IL-17A related inflammatory disease.

20. Use of the dsRNA molecule according to any one of claims 1 to 11 or the expression vector according to claim 12 or the pharmaceutical composition according to claim 13 for the manufacture of a medicament for the treatment of an inflammatory disease, in particular a pro-inflammatory cytokine such as TNF-α, IL-6 and/or IL-17A related inflammatory disease, in a subject in need thereof.

21. The method of claim 18 or 19, or the use of claim 20, wherein the inflammatory disease is selected from the group consisting of inflammatory bowel disease, inflammation caused by infection, inflammation caused by injury, inflammation of the respiratory system, and inflammation associated with cancer.

22. The method of claim 18 or 19, or the use of claim 20, wherein the inflammatory disease is selected from the group consisting of systemic lupus erythematosus, Crohn’s disease, psoriasis, colitis, ileitis, glomerulonephritis, asthma, dermatitis (including contact dermatitis and atopic dermatitis), vasculitis, chronic bronchitis, chronic prostatitis, appendicitis, pancreatitis, pelvic inflammation, polymyositis, and chronic obstructive pulmonary disease.

23. A method of treating a bone metabolism-related disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the dsRNA molecule of any one of claims 1-11 or the expression vector of claim 12 or the pharmaceutical composition of claim 13.

24. The method of claim 23, further comprising administering to the subject an additional therapeutic agent for treating a bone metabolism-related disorder.

25. Use of the dsRNA molecule of any one of claims 1-11 or the expression vector of claim 12 or the pharmaceutical composition of claim 13 for the manufacture of a medicament for the treatment of a bone metabolism-related disorder in a subject in need thereof.

26. The method of claim 23 or 24, or the use of claim 25, wherein the bone metabolism-related disorder is selected from the group consisting of osteomalacia, bone deficiency, osteolytic bone disease, renal bone disease, osteogenesis imperfecta, and bone destruction caused by cancer bone metastases.

siRNA	sense strand(5'-3')	antisense strand(5'-3')
si-TD029	CGCCCGAGAAGGUCGGCUGTT	CAGCCGACCUUCUCGGGCGTT
si-TD030	GCCCGAGAAGGUCGGCUGGTT	CCAGCCGACCUUCUCGGGCTT
si-TD031	CCCGAGAAGGUCGGCUGGGTT	CCCAGCCGACCUUCUCGGGTT
si-TD032	CCGAGAAGGUCGGCUGGGUTT	ACCCAGCCGACCUUCUCGGTT
si-TD033	CGAGAAGGUCGGCUGGGUCTT	GACCCAGCCGACCUUCUCGTT
si-TD034	GAGAAGGUCGGCUGGGUCCTT	GGACCCAGCCGACCUUCUCTT
si-TD035	AGAAGGUCGGCUGGGUCCGTT	CGGACCCAGCCGACCUUCUTT
si-TD036	GAAGGUCGGCUGGGUCCGGTT	CCGGACCCAGCCGACCUUCTT
si-TD037	AAGGUCGGCUGGGUCCGGATT	UCCGGACCCAGCCGACCUUTT
si-TD038	AGGUCGGCUGGGUCCGGAATT	UUCCGGACCCAGCCGACCUTT
si-TD040	GUCGGCUGGGUCCGGAAAUTT	AUUUCCGGACCCAGCCGACTT
si-TD041	UCGGCUGGGUCCGGAAAUUTT	AAUUUCCGGACCCAGCCGATT
si-TD042	CGGCUGGGUCCGGAAAUUCTT	GAAUUUCCGGACCCAGCCGTT
si-TD043	GGCUGGGUCCGGAAAUUCUTT	AGAAUUUCCGGACCCAGCCTT
si-TD044	GCUGGGUCCGGAAAUUCUGTT	CAGAAUUUCCGGACCCAGCTT
si-TD045	CUGGGUCCGGAAAUUCUGCTT	GCAGAAUUUCCGGACCCAGTT
si-TD046	UGGGUCCGGAAAUUCUGCGTT	CGCAGAAUUUCCGGACCCATT
si-TD047	GGGUCCGGAAAUUCUGCGGTT	CCGCAGAAUUUCCGGACCCTT
si-TD048	GGUCCGGAAAUUCUGCGGGTT	CCCGCAGAAUUUCCGGACCTT
si-TD049	GUCCGGAAAUUCUGCGGGATT	UCCCGCAGAAUUUCCGGACTT
si-TD050	UCCGGAAAUUCUGCGGGAATT	UUCCCGCAGAAUUUCCGGATT
si-TD052	CGGAAAUUCUGCGGGAAAGTT	CUUUCCCGCAGAAUUUCCGTT
si-TD053	GGAAAUUCUGCGGGAAAGGTT	CCUUUCCCGCAGAAUUUCCTT
si-TD054	GAAAUUCUGCGGGAAAGGGTT	CCCUUUCCCGCAGAAUUUCTT
si-TD055	AAAUUCUGCGGGAAAGGGATT	UCCCUUUCCCGCAGAAUUUTT
si-TD056	AAUUCUGCGGGAAAGGGAUTT	AUCCCUUUCCCGCAGAAUUTT
si-TD057	AUUCUGCGGGAAAGGGAUUTT	AAUCCCUUUCCCGCAGAAUTT
si-TD058	UUCUGCGGGAAAGGGAUUUTT	AAAUCCCUUUCCCGCAGAATT
si-TD059	UCUGCGGGAAAGGGAUUUUTT	AAAAUCCCUUUCCCGCAGATT
si-TD060	CUGCGGGAAAGGGAUUUUCTT	GAAAAUCCCUUUCCCGCAGTT
si-TD061	UGCGGGAAAGGGAUUUUCATT	UGAAAAUCCCUUUCCCGCATT
si-TD062	GCGGGAAAGGGAUUUUCAGTT	CUGAAAAUCCCUUUCCCGCTT
si-TD063	CGGGAAAGGGAUUUUCAGGTT	CCUGAAAAUCCCUUUCCCGTT
si-TD064	GGGAAAGGGAUUUUCAGGGTT	CCCUGAAAAUCCCUUUCCCTT
si-TD065	GGAAAGGGAUUUUCAGGGATT	UCCCUGAAAAUCCCUUUCCTT
si-TD066	GAAAGGGAUUUUCAGGGAGTT	CUCCCUGAAAAUCCCUUUCTT
si-TD067	AAAGGGAUUUUCAGGGAGATT	UCUCCCUGAAAAUCCCUUUTT
si-TD068	AAGGGAUUUUCAGGGAGAUTT	AUCUCCCUGAAAAUCCCUUTT
si-TD069	AGGGAUUUUCAGGGAGAUUTT	AAUCUCCCUGAAAAUCCCUTT
si-TD070	GGGAUUUUCAGGGAGAUUUTT	AAAUCUCCCUGAAAAUCCCTT
si-TD071	GGAUUUUCAGGGAGAUUUGTT	CAAAUCUCCCUGAAAAUCCTT
si-TD072	GAUUUUCAGGGAGAUUUGGTT	CCAAAUCUCCCUGAAAAUCTT
si-TD073	AUUUUCAGGGAGAUUUGGATT	UCCAAAUCUCCCUGAAAAUTT
si-TD074	UUUUCAGGGAGAUUUGGAATT	UUCCAAAUCUCCCUGAAAATT

Figure 1

si-TD075	UUUCAGGGAGAUUUGGAAATT	UUUCCAAAUCUCCCUGAAATT
si-TD076	UUCAGGGAGAUUUGGAAAATT	UUUUCCAAAUCUCCCUGAATT
si-TD077	UCAGGGAGAUUUGGAAAAATT	UUUUUCCAAAUCUCCCUGATT
si-TD078	CAGGGAGAUUUGGAAAAACTT	GUUUUUCCAAAUCUCCCUGTT
si-TD079	AGGGAGAUUUGGAAAAACCTT	GGUUUUUCCAAAUCUCCCUTT
si-TD080	GGGAGAUUUGGAAAAACCGTT	CGGUUUUUCCAAAUCUCCCTT
si-TD081	GGAGAUUUGGAAAAACCGCTT	GCGGUUUUUCCAAAUCUCCTT
si-TD082	GAGAUUUGGAAAAACCGCUTT	AGCGGUUUUUCCAAAUCUCTT
si-TD083	AGAUUUGGAAAAACCGCUATT	UAGCGGUUUUUCCAAAUCUTT
si-TD084	GAUUUGGAAAAACCGCUAUTT	AUAGCGGUUUUUCCAAAUCTT
si-TD085	AUUUGGAAAAACCGCUAUGTT	CAUAGCGGUUUUUCCAAAUTT
si-TD086	UUUGGAAAAACCGCUAUGUTT	ACAUAGCGGUUUUUCCAAATT
si-TD087	UUGGAAAAACCGCUAUGUGTT	CACAUAGCGGUUUUUCCAATT
si-TD088	UGGAAAAACCGCUAUGUGGTT	CCACAUAGCGGUUUUUCCATT
si-TD089	GGAAAAACCGCUAUGUGGUTT	ACCACAUAGCGGUUUUUCCTT
si-TD090	GAAAAACCGCUAUGUGGUGTT	CACCACAUAGCGGUUUUUCTT
si-TD091	AAAAACCGCUAUGUGGUGCTT	GCACCACAUAGCGGUUUUUTT
si-TD092	AAAACCGCUAUGUGGUGCUTT	AGCACCACAUAGCGGUUUUTT
si-TD093	AAACCGCUAUGUGGUGCUGTT	CAGCACCACAUAGCGGUUUTT
si-TD094	AACCGCUAUGUGGUGCUGATT	UCAGCACCACAUAGCGGUUTT
si-TD096	CCGCUAUGUGGUGCUGAAATT	UUUCAGCACCACAUAGCGGTT
si-TD097	CGCUAUGUGGUGCUGAAAGTT	CUUUCAGCACCACAUAGCGTT
si-TD098	GCUAUGUGGUGCUGAAAGGTT	CCUUUCAGCACCACAUAGCTT
si-TD136	GAGAAGGAGGUAAAAGAUGTT	CAUCUUUUACCUCCUUCUCTT
si-TD137	AGAAGGAGGUAAAAGAUGATT	UCAUCUUUUACCUCCUUCUTT
si-TD138	GAAGGAGGUAAAAGAUGAGTT	CUCAUCUUUUACCUCCUUCTT
si-TD139	AAGGAGGUAAAAGAUGAGATT	UCUCAUCUUUUACCUCCUUTT
si-TD140	AGGAGGUAAAAGAUGAGAATT	UUCUCAUCUUUUACCUCCUTT
si-TD141	GGAGGUAAAAGAUGAGAAATT	UUUCUCAUCUUUUACCUCCTT
si-TD142	GAGGUAAAAGAUGAGAAAATT	UUUUCUCAUCUUUUACCUCTT
si-TD143	AGGUAAAAGAUGAGAAAAATT	UUUUUCUCAUCUUUUACCUTT
si-TD182	UGAGUGACUAUGAGAAGUGTT	CACUUCUCAUAGUCACUCATT
si-TD181	CUGAGUGACUAUGAGAAGUTT	ACUUCUCAUAGUCACUCAGTT
si-TD179	ACCUGAGUGACUAUGAGAATT	UUCUCAUAGUCACUCAGGUTT
si-TD178	GACCUGAGUGACUAUGAGATT	UCUCAUAGUCACUCAGGUCTT
si-TD177	UGACCUGAGUGACUAUGAGTT	CUCAUAGUCACUCAGGUCATT
si-TD176	UUGACCUGAGUGACUAUGATT	UCAUAGUCACUCAGGUCAATT
si-TD175	UUUGACCUGAGUGACUAUGTT	CAUAGUCACUCAGGUCAAATT
si-TD224	GCAGGAGCAAGAAAAAUCATT	UGAUUUUUCUUGCUCCUGCTT
si-TD223	AGCAGGAGCAAGAAAAAUCTT	GAUUUUUCUUGCUCCUGCUTT
si-TD222	GAGCAGGAGCAAGAAAAAUTT	AUUUUUCUUGCUCCUGCUCTT
si-TD221	AGAGCAGGAGCAAGAAAAATT	UUUUUCUUGCUCCUGCUCUTT
si-TD220	AAGAGCAGGAGCAAGAAAATT	UUUUCUUGCUCCUGCUCUUTT
si-TD219	CAAGAGCAGGAGCAAGAAATT	UUUCUUGCUCCUGCUCUUGTT
si-TD218	CCAAGAGCAGGAGCAAGAATT	UUCUUGCUCCUGCUCUUGGTT

Figure 1(continued)

si-TD217	UCCAAGAGCAGGAGCAAGATT	UCUUGCUCCUGCUCUUGGATT
si-TD385	AGGACAGCUAUCUUGCCCATT	UGGGCAAGAUAGCUGUCCUTT
si-TD384	GAGGACAGCUAUCUUGCCCTT	GGGCAAGAUAGCUGUCCUCTT
si-TD383	GGAGGACAGCUAUCUUGCCTT	GGCAAGAUAGCUGUCCUCCTT
si-TD382	AGGAGGACAGCUAUCUUGCTT	GCAAGAUAGCUGUCCUCCUTT
si-TD381	GAGGAGGACAGCUAUCUUGTT	CAAGAUAGCUGUCCUCCUCTT
si-TD380	UGAGGAGGACAGCUAUCUUTT	AAGAUAGCUGUCCUCCUCATT
si-TD379	UUGAGGAGGACAGCUAUCUTT	AGAUAGCUGUCCUCCUCAATT
si-TD378	GUUGAGGAGGACAGCUAUCTT	GAUAGCUGUCCUCCUCAACTT
si-TD377	CGUUGAGGAGGACAGCUAUTT	AUAGCUGUCCUCCUCAACGTT
si-TD376	CCGUUGAGGAGGACAGCUATT	UAGCUGUCCUCCUCAACGGTT
si-TD375	ACCGUUGAGGAGGACAGCUTT	AGCUGUCCUCCUCAACGGUTT
si-TD374	CACCGUUGAGGAGGACAGCTT	GCUGUCCUCCUCAACGGUGTT
si-TD373	UCACCGUUGAGGAGGACAGTT	CUGUCCUCCUCAACGGUGATT
si-TD372	GUCACCGUUGAGGAGGACATT	UGUCCUCCUCAACGGUGACTT
si-TD371	GGUCACCGUUGAGGAGGACTT	GUCCUCCUCAACGGUGACCTT
si-TD370	AGGUCACCGUUGAGGAGGATT	UCCUCCUCAACGGUGACCUTT
si-TD369	GAGGUCACCGUUGAGGAGGTT	CCUCCUCAACGGUGACCUCTT
si-TD368	UGAGGUCACCGUUGAGGAGTT	CUCCUCAACGGUGACCUCATT
si-TD367	AUGAGGUCACCGUUGAGGATT	UCCUCAACGGUGACCUCAUTT
si-TD366	GAUGAGGUCACCGUUGAGGTT	CCUCAACGGUGACCUCAUCTT
si-TD364	UGGAUGAGGUCACCGUUGATT	UCAACGGUGACCUCAUCCATT
si-TD363	UUGGAUGAGGUCACCGUUGTT	CAACGGUGACCUCAUCCAATT
si-TD362	CUUGGAUGAGGUCACCGUUTT	AACGGUGACCUCAUCCAAGTT
si-TD361	UCUUGGAUGAGGUCACCGUTT	ACGGUGACCUCAUCCAAGATT
si-TD360	AUCUUGGAUGAGGUCACCGTT	CGGUGACCUCAUCCAAGAUTT
si-TD359	UAUCUUGGAUGAGGUCACCTT	GGUGACCUCAUCCAAGAUATT
si-TD358	GUAUCUUGGAUGAGGUCACTT	GUGACCUCAUCCAAGAUACTT
si-TD357	CGUAUCUUGGAUGAGGUCATT	UGACCUCAUCCAAGAUACGTT
si-TD356	CCGUAUCUUGGAUGAGGUCTT	GACCUCAUCCAAGAUACGGTT
si-TD355	ACCGUAUCUUGGAUGAGGUTT	ACCUCAUCCAAGAUACGGUTT
si-TD354	AACCGUAUCUUGGAUGAGGTT	CCUCAUCCAAGAUACGGUUTT
si-TD454	AAGACCCUUCCCCUGAGGATT	UCCUCAGGGGAAGGGUCUUTT
si-TD453	GAAGACCCUUCCCCUGAGGTT	CCUCAGGGGAAGGGUCUUCTT
si-TD452	GGAAGACCCUUCCCCUGAGTT	CUCAGGGGAAGGGUCUUCCTT
si-TD451	AGGAAGACCCUUCCCCUGATT	UCAGGGGAAGGGUCUUCCUTT
si-TD450	GAGGAAGACCCUUCCCCUGTT	CAGGGGAAGGGUCUUCCUCTT
si-TD449	AGAGGAAGACCCUUCCCCUTT	AGGGGAAGGGUCUUCCUCUTT
si-TD448	AAGAGGAAGACCCUUCCCCTT	GGGGAAGGGUCUUCCUCUUTT
si-TD447	CAAGAGGAAGACCCUUCCCTT	GGGAAGGGUCUUCCUCUUGTT
si-TD446	CCAAGAGGAAGACCCUUCCTT	GGAAGGGUCUUCCUCUUGGTT
si-TD445	UCCAAGAGGAAGACCCUUCTT	GAAGGGUCUUCCUCUUGGATT
si-TD444	AUCCAAGAGGAAGACCCUUTT	AAGGGUCUUCCUCUUGGAUTT
si-TD443	GAUCCAAGAGGAAGACCCUTT	AGGGUCUUCCUCUUGGAUCTT
si-TD442	UGAUCCAAGAGGAAGACCCTT	GGGUCUUCCUCUUGGAUCATT

Figure 1(continued)

si-TD509	GGACAAGUCUGUGGCCCAGTT	CUGGGCCACAGACUUGUCCTT
si-TD508	UGGACAAGUCUGUGGCCCATT	UGGGCCACAGACUUGUCCATT
si-TD507	CUGGACAAGUCUGUGGCCCTT	GGGCCACAGACUUGUCCAGTT
si-TD577	GCCUCCCUGGAGGAGAUCCTT	GGAUCUCCUCCAGGGAGGCTT
si-TD578	CCUCCCUGGAGGAGAUCCUTT	AGGAUCUCCUCCAGGGAGGTT
si-TD611	GGUAGCAAGGAAACUGGAGTT	CUCCAGUUUCCUUGCUACCTT
si-TD610	UGGUAGCAAGGAAACUGGATT	UCCAGUUUCCUUGCUACCATT
si-TD609	CUGGUAGCAAGGAAACUGGTT	CCAGUUUCCUUGCUACCAGTT
si-TD608	CCUGGUAGCAAGGAAACUGTT	CAGUUUCCUUGCUACCAGGTT
si-TD607	ACCUGGUAGCAAGGAAACUTT	AGUUUCCUUGCUACCAGGUTT
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si-TD604	AGGACCUGGUAGCAAGGAATT	UUCCUUGCUACCAGGUCCUTT
si-TD603	CAGGACCUGGUAGCAAGGATT	UCCUUGCUACCAGGUCCUGTT
si-TD602	CCAGGACCUGGUAGCAAGGTT	CCUUGCUACCAGGUCCUGGTT
si-TD601	UCCAGGACCUGGUAGCAAGTT	CUUGCUACCAGGUCCUGGATT
si-TD600	AUCCAGGACCUGGUAGCAATT	UUGCUACCAGGUCCUGGAUTT
si-TD599	GAUCCAGGACCUGGUAGCATT	UGCUACCAGGUCCUGGAUCTT
si-TD598	GGAUCCAGGACCUGGUAGCTT	GCUACCAGGUCCUGGAUCCTT
si-TD597	CGGAUCCAGGACCUGGUAGTT	CUACCAGGUCCUGGAUCCGTT
si-TD596	CCGGAUCCAGGACCUGGUATT	UACCAGGUCCUGGAUCCGGTT
si-TD595	CCCGGAUCCAGGACCUGGUTT	ACCAGGUCCUGGAUCCGGGTT
si-TD594	UCCCGGAUCCAGGACCUGGTT	CCAGGUCCUGGAUCCGGGATT
si-TD593	GUCCCGGAUCCAGGACCUGTT	CAGGUCCUGGAUCCGGGACTT
si-TD592	UGUCCCGGAUCCAGGACCUTT	AGGUCCUGGAUCCGGGACATT
si-TD591	CUGUCCCGGAUCCAGGACCTT	GGUCCUGGAUCCGGGACAGTT
si-TD590	GCUGUCCCGGAUCCAGGACTT	GUCCUGGAUCCGGGACAGCTT
si-TD589	AGCUGUCCCGGAUCCAGGATT	UCCUGGAUCCGGGACAGCUTT
si-TD588	CAGCUGUCCCGGAUCCAGGTT	CCUGGAUCCGGGACAGCUGTT
si-TD587	GCAGCUGUCCCGGAUCCAGTT	CUGGAUCCGGGACAGCUGCTT
si-TD586	GGCAGCUGUCCCGGAUCCATT	UGGAUCCGGGACAGCUGCCTT
si-TD585	GGGCAGCUGUCCCGGAUCCTT	GGAUCCGGGACAGCUGCCCTT
si-TD721	AGCUGAGAGACCUGUACAGTT	CUGUACAGGUCUCUCAGCUTT
si-TD720	GAGCUGAGAGACCUGUACATT	UGUACAGGUCUCUCAGCUCTT
si-TD719	GGAGCUGAGAGACCUGUACTT	GUACAGGUCUCUCAGCUCCTT
si-TD718	GGGAGCUGAGAGACCUGUATT	UACAGGUCUCUCAGCUCCCTT
si-TD742	AGAUGGACCUGCAGACCCCTT	GGGGUCUGCAGGUCCAUCUTT
si-TD741	CAGAUGGACCUGCAGACCCTT	GGGUCUGCAGGUCCAUCUGTT
si-TD744	ACUCCCACCUCAGACAGACTT	GUCUGUCUGAGGUGGGAGUTT
si-TD743	GACUCCCACCUCAGACAGATT	UCUGUCUGAGGUGGGAGUCTT
si-TD144	GGUAAAAGAUGAGAAAAAUTT	AUUUUUCUCAUCUUUUACCTT
si-TD145	GUAAAAGAUGAGAAAAAUATT	UAUUUUUCUCAUCUUUUACTT
si-TD479	CUCUUGUGCUGAGAGCUUUTT	AAAGCUCUCAGCACAAGAGTT
si-TD480	UCUUGUGCUGAGAGCUUUCTT	GAAAGCUCUCAGCACAAGATT
si-TD481	CUUGUGCUGAGAGCUUUCGTT	CGAAAGCUCUCAGCACAAGTT
si-TD482	UUGUGCUGAGAGCUUUCGGTT	CCGAAAGCUCUCAGCACAATT

Figure 1(continued)

si-TD483	UGUGCUGAGAGCUUUCGGGTT	CCCGAAAGCUCUCAGCACATT
si-TD584	GGGGCAGCUGUCCCGGAUCTT	GAUCCGGGACAGCUGCCCCTT
si-TD583	CGGGGCAGCUGUCCCGGAUTT	AUCCGGGACAGCUGCCCCGTT
si-TD582	CCGGGGCAGCUGUCCCGGATT	UCCGGGACAGCUGCCCCGGTT
si-TD740	ACAGAUGGACCUGCAGACCTT	GGUCUGCAGGUCCAUCUGUTT
si-TD739	GACAGAUGGACCUGCAGACTT	GUCUGCAGGUCCAUCUGUCTT
si-TD738	AGACAGAUGGACCUGCAGATT	UCUGCAGGUCCAUCUGUCUTT
si-TD737	CAGACAGAUGGACCUGCAGTT	CUGCAGGUCCAUCUGUCUGTT
si-TD736	ACAGACAGAUGGACCUGCATT	UGCAGGUCCAUCUGUCUGUTT
si-TD735	UACAGACAGAUGGACCUGCTT	GCAGGUCCAUCUGUCUGUATT
si-TD734	GUACAGACAGAUGGACCUGTT	CAGGUCCAUCUGUCUGUACTT
si-TD733	UGUACAGACAGAUGGACCUTT	AGGUCCAUCUGUCUGUACATT
si-TD732	CUGUACAGACAGAUGGACCTT	GGUCCAUCUGUCUGUACAGTT
si-TD731	CCUGUACAGACAGAUGGACTT	GUCCAUCUGUCUGUACAGGTT
si-TD730	ACCUGUACAGACAGAUGGATT	UCCAUCUGUCUGUACAGGUTT
si-TD729	GACCUGUACAGACAGAUGGTT	CCAUCUGUCUGUACAGGUCTT
si-TD728	AGACCUGUACAGACAGAUGTT	CAUCUGUCUGUACAGGUCUTT
si-TD727	GAGACCUGUACAGACAGAUTT	AUCUGUCUGUACAGGUCUCTT
si-TD726	AGAGACCUGUACAGACAGATT	UCUGUCUGUACAGGUCUCUTT
si-TD725	GAGAGACCUGUACAGACAGTT	CUGUCUGUACAGGUCUCUCTT
si-TD723	CUGAGAGACCUGUACAGACTT	GUCUGUACAGGUCUCUCAGTT
si-TD722	GCUGAGAGACCUGUACAGATT	UCUGUACAGGUCUCUCAGCTT
si-TD717	AGGGAGCUGAGAGACCUGUTT	ACAGGUCUCUCAGCUCCCUTT
si-TD716	CAGGGAGCUGAGAGACCUGTT	CAGGUCUCUCAGCUCCCUGTT
si-TD715	UCAGGGAGCUGAGAGACCUTT	AGGUCUCUCAGCUCCCUGATT
si-TD714	GUCAGGGAGCUGAGAGACCTT	GGUCUCUCAGCUCCCUGACTT
si-7	UGGGAGAUGGGAAGCGAAATT	UUUCGCUUCCCAUCUCCCATT
si-10	CAGACAAAGGGGCCACCUATT	UAGGUGGCCCCUUUGUCUGTT
si-1	GGACCUGGUAGCAAGGAAATT	UUUCCUUGCUACCAGGUCCTT
NC-1	GAAAGAUAGAGAAGGUAGATT	UCUACCUUCUCUAUCUUUCTT
NC-2	GCAACAGCCGAUGAAGUUATT	UAACUUCAUCGGCUGUUGCTT
NC-3	GGCCGAGCAACGAAUGUCATT	UGACAUUCGUUGCUCGGCCTT
NC-4	GGACAUCGAACGAAGUGCUTT	AGCACUUCGUUCGAUGUCCTT
NC-5	GCGGUCCCUGCGACGUACATT	UGUACGUCGCAGGGACCGCTT
NC-6	GCUGCGCGAACCCAUCAAATT	UUUGAUGGGUUCGCGCAGCTT
NC-7	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
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Figure 1(continued)

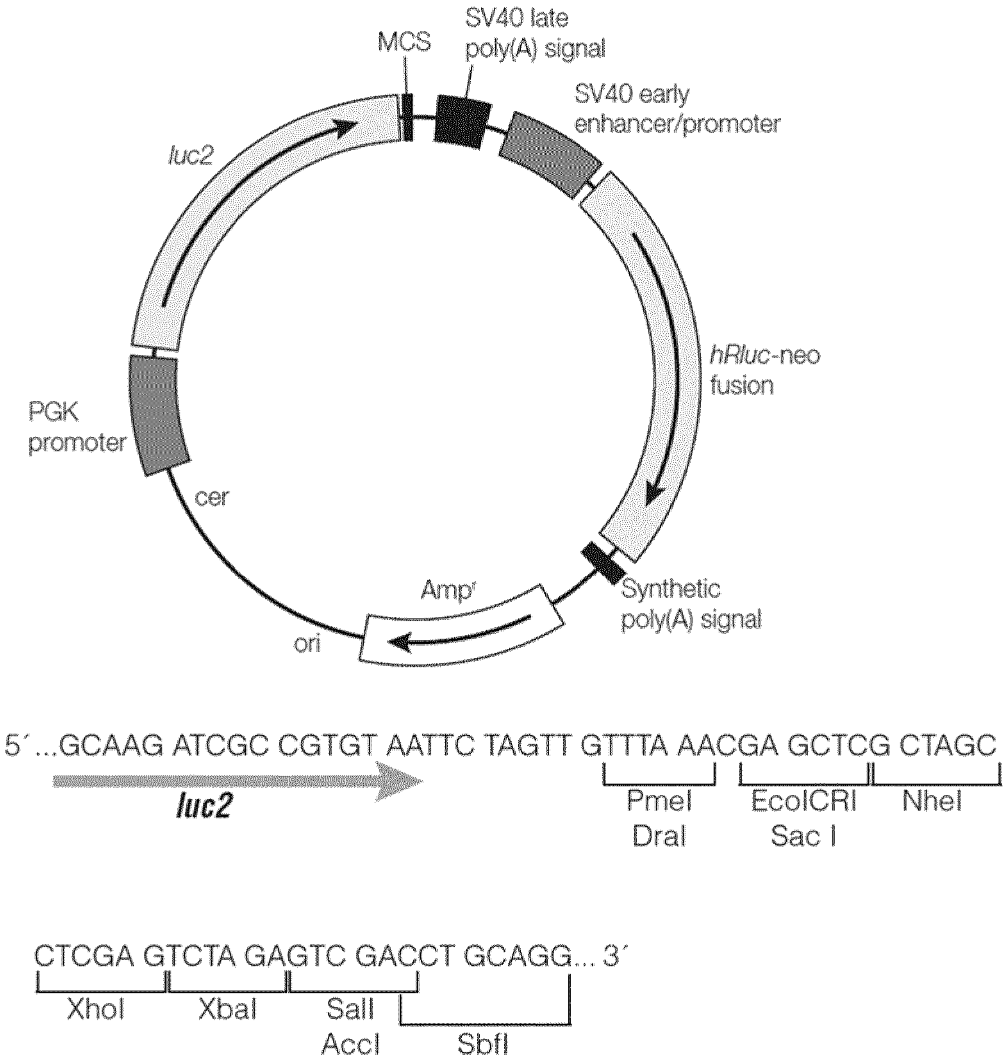
MCS
SV40 late poly(A) signal
SV40 early enhancer/promoter
luc2
hRluc-neo fusion
PGK promoter
cer
Ampʳ
ori
Synthetic poly(A) signal
5´...GCAAG ATCGC CGTGT AATTC TAGTT GTTTA AACGA GCTCG CTAGC
luc2
PmeI
DraI
EcoICRI
Sac I
NheI
CTCGA GTCTA GAGTC GACCT GCAGG... 3´
XhoI
XbaI
SalI
AccI
SbfI

Figure 2

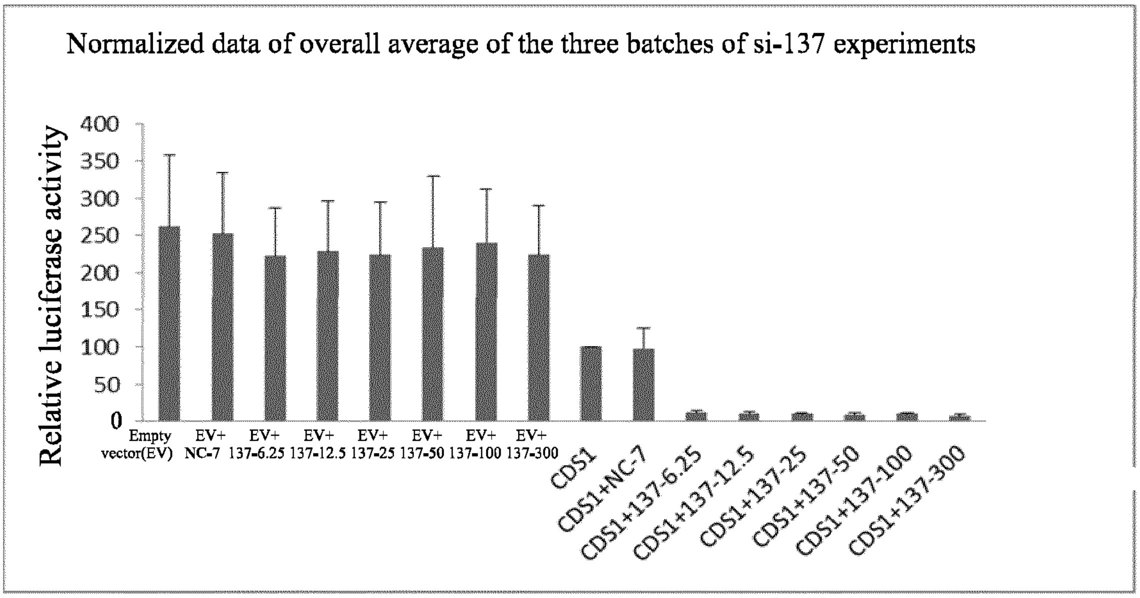
Normalized data of overall average of the three batches of si-137 experiments
Relative luciferase activity
400
350
300
250
200
150
100
50
0
Empty vector(EV)
EV+ NC-7
EV+ 137-6.25
EV+ 137-12.5
EV+ 137-25
EV+ 137-50
EV+ 137-100
EV+ 137-300
CDS1
CDS1+NC-7
CDS1+137-6.25
CDS1+137-12.5
CDS1+137-25
CDS1+137-50
CDS1+137-100
CDS1+137-300

Figure 3

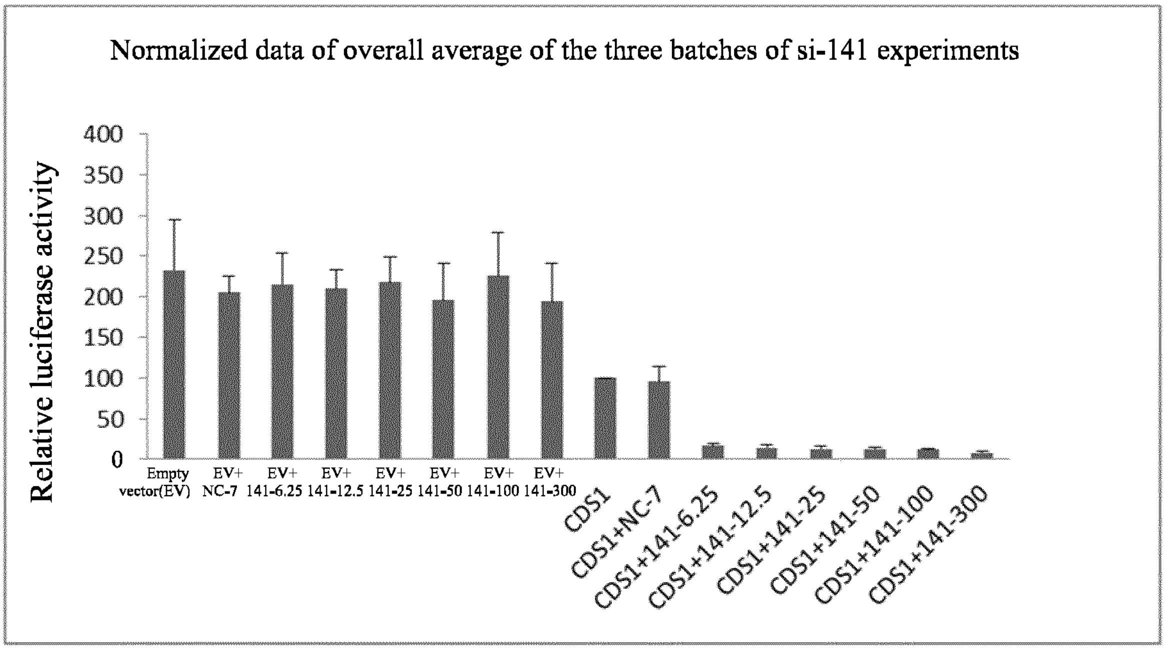
Normalized data of overall average of the three batches of si-141 experiments
Relative luciferase activity
400
350
300
250
200
150
100
50
0
Empty vector(EV)
EV+ NC-7
EV+ 141-6.25
EV+ 141-12.5
EV+ 141-25
EV+ 141-50
EV+ 141-100
EV+ 141-300
CDS1
CDS1+NC-7
CDS1+141-6.25
CDS1+141-12.5
CDS1+141-25
CDS1+141-50
CDS1+141-100
CDS1+141-300

Figure 4

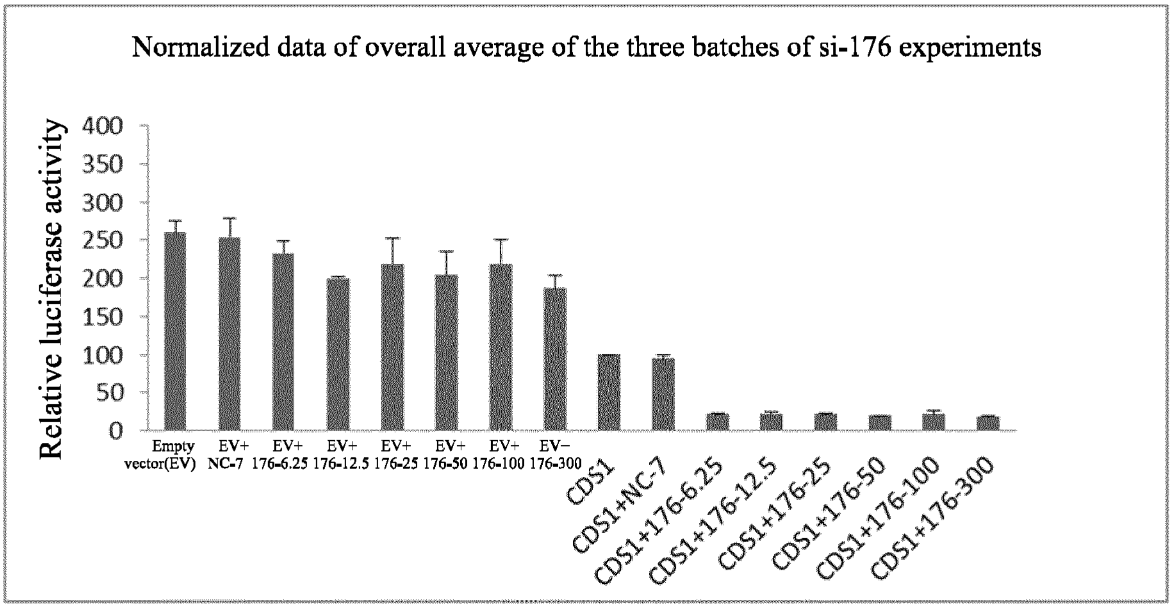
Normalized data of overall average of the three batches of si-176 experiments
Relative luciferase activity
400
350
300
250
200
150
100
50
0
Empty vector(EV)
EV+ NC-7
EV+ 176-6.25
EV+ 176-12.5
EV+ 176-25
EV+ 176-50
EV+ 176-100
EV– 176-300
CDS1
CDS1+NC-7
CDS1+176-6.25
CDS1+176-12.5
CDS1+176-25
CDS1+176-50
CDS1+176-100
CDS1+176-300

Figure 5

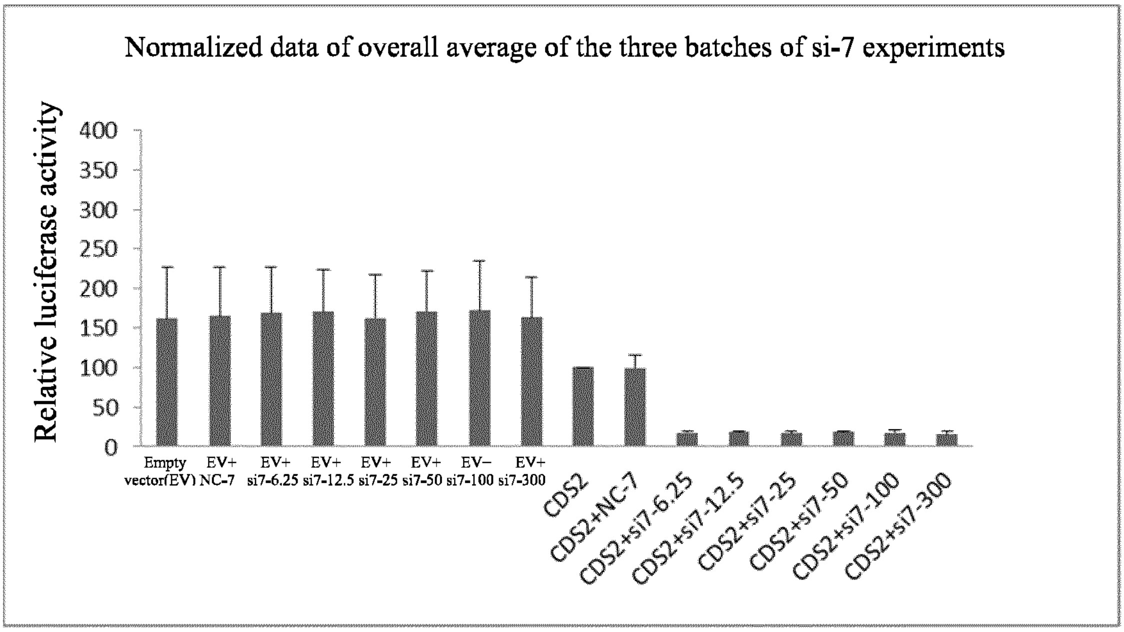
Normalized data of overall average of the three batches of si-7 experiments
Relative luciferase activity
400
350
300
250
200
150
100
50
0
Empty vector(EV)
EV+ NC-7
EV+ si7-6.25
EV+ si7-12.5
EV+ si7-25
EV+ si7-50
EV− si7-100
EV+ si7-300
CDS2
CDS2+NC-7
CDS2+si7-6.25
CDS2+si7-12.5
CDS2+si7-25
CDS2+si7-50
CDS2+si7-100
CDS2+si7-300

Figure 6

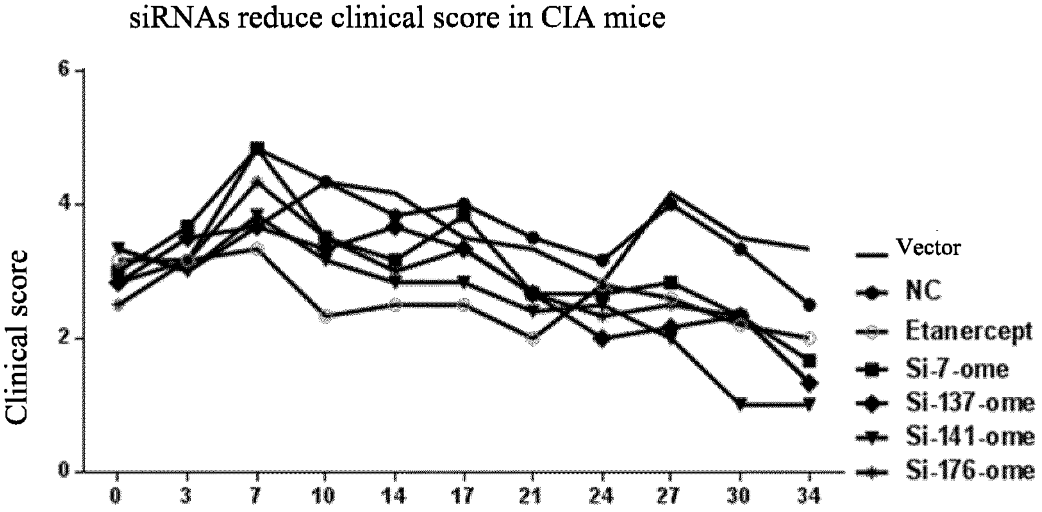
siRNAs reduce clinical score in CIA mice
Clinical score
6
4
2
0
0
3
7
10
14
17
21
24
27
30
34
Vector
NC
Etanercept
Si-7-ome
Si-137-ome
Si-141-ome
Si-176-ome

Figure 7

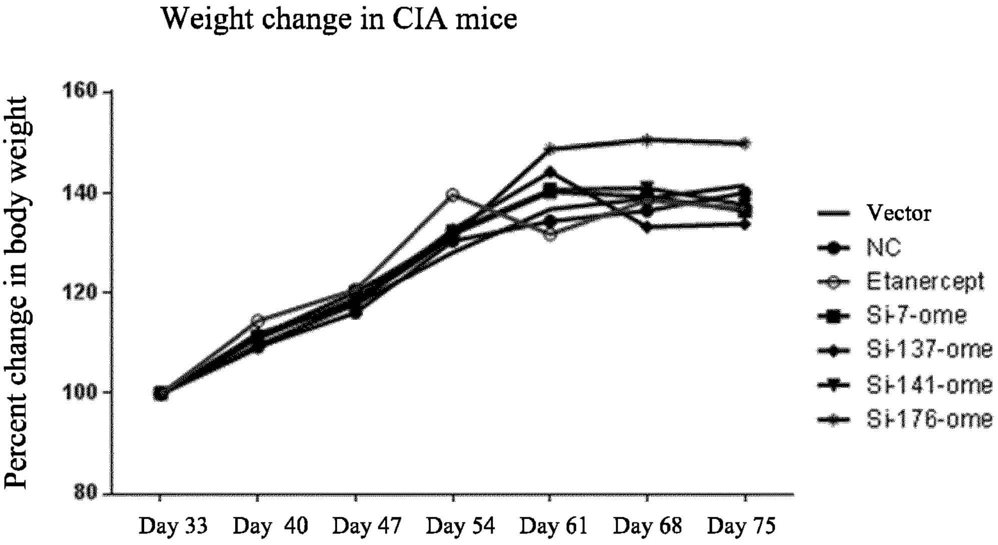
Weight change in CIA mice
Percent change in body weight
160
140
120
100
80
Day 33
Day 40
Day 47
Day 54
Day 61
Day 68
Day 75
Vector
NC
Etanercept
Si-7-ome
Si-137-ome
Si-141-ome
Si-176-ome

Figure 8

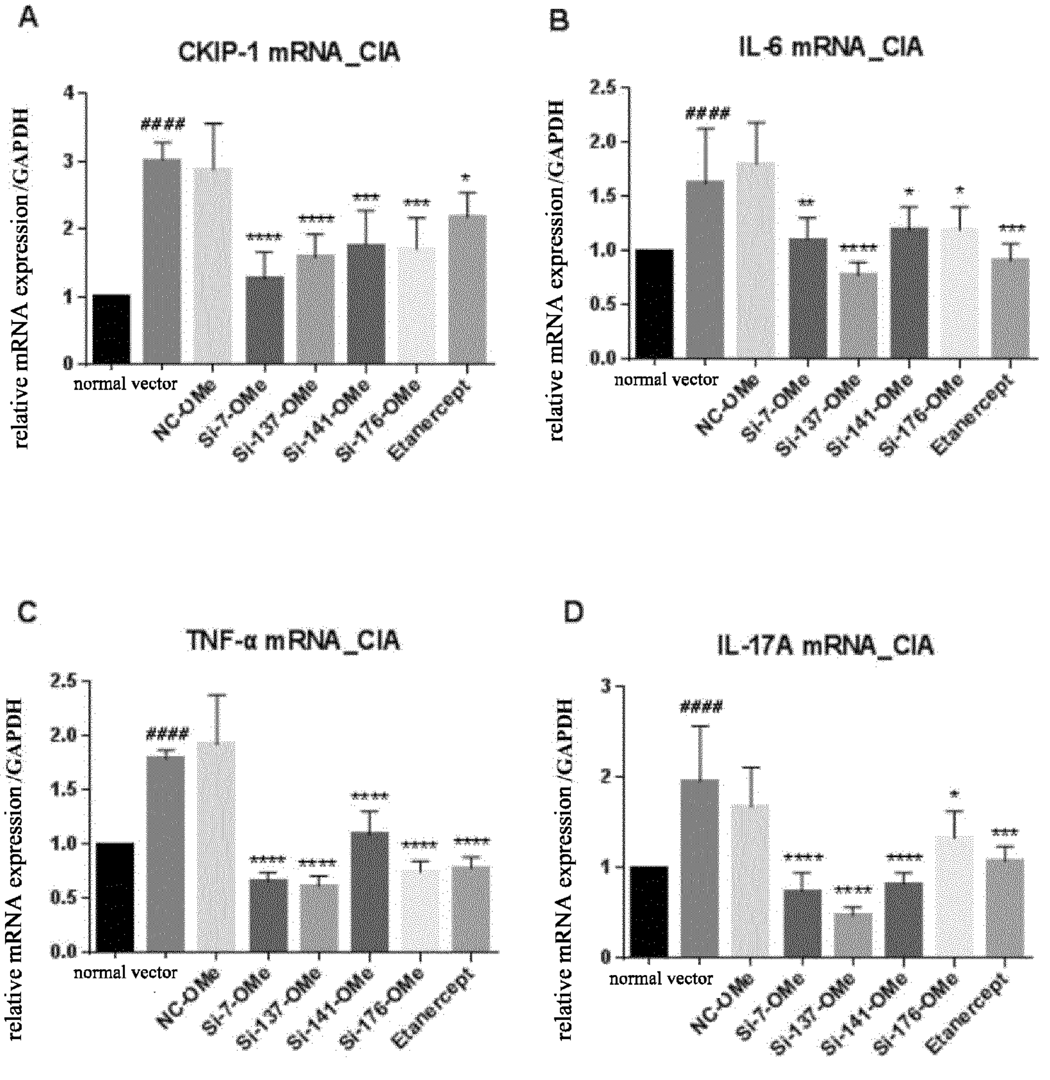
A
CKIP-1 mRNA_CIA
relative mRNA expression/GAPDH
0
1
2
3
4
####
****
****
***
***
*
normal vector
NC-OMe
Si-7-OMe
Si-137-OMe
Si-141-OMe
Si-176-OMe
Etanercept
B
IL-6 mRNA_CIA
relative mRNA expression/GAPDH
0.0
0.5
1.0
1.5
2.0
2.5
####
**
****
*
*
***
normal vector
NC-OMe
Si-7-OMe
Si-137-OMe
Si-141-OMe
Si-176-OMe
Etanercept
C
TNF-α mRNA_CIA
relative mRNA expression/GAPDH
0.0
0.5
1.0
1.5
2.0
2.5
####
****
****
****
****
****
normal vector
NC-OMe
Si-7-OMe
Si-137-OMe
Si-141-OMe
Si-176-OMe
Etanercept
D
IL-17A mRNA_CIA
relative mRNA expression/GAPDH
0
1
2
3
####
****
****
****
*
***
normal vector
NC-OMe
Si-7-OMe
Si-137-OMe
Si-141-OMe
Si-176-OMe
Etanercept

Figure 9

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

C12N 15/11(2006.01)i；C12N 15/63(2006.01)i；A61K 48/00(2006.01)i；A61P 19/02(2006.01)i；A61P 19/08(2006.01)i；A61P 19/10(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N; A61K; A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS, VEN (DWPI+SIPOABS), CNTXT, EPTXT, USTXT, WOTXT, CNKI, 百度学术, BAIDU SCHOLAR, ISI-WEB OF SCIENCE, Genbank+EMBL, 中国专利生物序列检索系统, NATIONAL BIO-SEQUENCE DATABASE OF CHINESE PATENT: 酪蛋白激酶, 关节炎, 炎症, 双链RNA, 干扰RNA, 发夹RNA, casein kinase, CKIP-1, dsRNA, double stranded RNA, double strand RNA, interfering RNA, siRNA, hairpin RNA, shRNA, arthritis, inflammation, search for SEQ ID NO: 63-64, 71-72, 83-84 and 161-162

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2213738 A2 (DHARMACON INC.) 04 August 2010 (2010-08-04) see abstract, and description, paragraphs [0011]-[0019], SEQ ID NO: 960335, 960351, 960354 and 960365	1-13
Y	EP 2213738 A2 (DHARMACON INC.) 04 August 2010 (2010-08-04) see abstract, and description, paragraphs [0011]-[0019], SEQ ID NO: 960335, 960351, 960354 and 960365	16, 20, 25
Y	WO 2015027895 A1 (SUZHOU RIBO LIFE SCIENCE CO., LTD.) 05 March 2015 (2015-03-05) see abstract, and description, page 1, line 32 to page 3, line 18 and page 9, lines 15-23 and embodiments 4 and 5 .	16, 20, 25

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search **23 November 2018**	Date of mailing of the international search report **05 December 2018**
Name and mailing address of the ISA/CN **State Intellectual Property Office of the P. R. China** **No. 6, Xitucheng Road, Jimenqiao Haidian District, Beijing 100088** **China**	Authorized officer
Facsimile No. **(86-10)62019451**	Telephone No.

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

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C.	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	陈俊凤等 (CHEN, Junfeng et al.). "MicroRNA-20a通过调节CKIP-1促进小鼠C3H/10T1/2成骨分化 (MicroRNA-20a Promotes Osteogenic Differentiation of C3H/10T1/2 Cells Through Regulating CKIP-1 Expression)" *中国实验血液学杂志 (Journal of Experimental Hematology),* Vol. 25, No. (1), 20 February 2017 (2017-02-20), ISSN: 1009-2137, see pages 214-220	1-13, 16, 20, 25
A	GUO, B. et al. "Therapeutic RNA Interference Targeting CKIP-1 with a Cross-species Sequence to Stimulate Bone Formation" *Bone,* Vol. vol. 59, 15 November 2013 (2013-11-15), ISSN: 8756-3282, see pages 76-88	1-13, 16, 20, 25

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **14-15, 17-19, 21-24, 26**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Claims 14-15, 17-19, 21-24 and 26 relate to a method for disposing a human or animal body by surgery or treatment.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

[1] Invention 1: claims 1-13, 16, 20 and 25 (all in part) relate to a dsRNA molecule comprising a sense strand shown in SEQ ID NO: 63 and an antisense strand shown in SEQ ID NO: 64, an expression vector comprising the dsRNA molecule, a pharmaceutical composition comprising the dsRNA molecule or the expression vector and a pharmaceutical use of the dsRNA molecule, the expression vector or the pharmaceutical composition;

[2] invention 2: claims 1-13, 16, 20 and 25 (all in part) relate to a dsRNA molecule comprising a sense strand shown in SEQ ID NO: 71 and an antisense strand shown in SEQ ID NO: 72, an expression vector comprising the dsRNA molecule, a pharmaceutical composition comprising the dsRNA molecule or the expression vector and a pharmaceutical use of the dsRNA molecule, the expression vector or the pharmaceutical composition;

[3] invention 3: claims 1-13, 16, 20 and 25 (all in part) relate to a dsRNA molecule comprising a sense strand shown in SEQ ID NO: 83 and an antisense strand shown in SEQ ID NO: 84, an expression vector comprising the dsRNA molecule, a pharmaceutical composition comprising the dsRNA molecule or the expression vector and a pharmaceutical use of the dsRNA molecule, the expression vector or the pharmaceutical composition; and

[4] invention 4: claims 1-13, 16, 20 and 25 (all in part) relate to a dsRNA molecule comprising a sense strand shown in SEQ ID NO: 161 and an antisense strand shown in SEQ ID NO: 162, an expression vector comprising the dsRNA molecule, a pharmaceutical composition comprising the dsRNA molecule or the expression vector and a pharmaceutical use of the dsRNA molecule, the expression vector or the pharmaceutical composition.

[5] Inventions 1-4 relate to dsRNA molecules of different structures, and thus do not share the same or corresponding special technical feature therebetween, do not have a technical relationship, do not fall within a single general inventive concept and do not comply with PCT Rule 13.1, 13.2 and 13.3.

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Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

1. ☑ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☑ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2018/104552

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
EP 2213738 A2	04 August 2010	US 2007088155 A1	19 April 2007
		US 2010291681 A1	18 November 2010
		US 2009298176 A1	03 December 2009
		US 2007207974 A1	06 September 2007
		US 2010075869 A1	25 March 2010
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		ES 2440284 T3	28 January 2014
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		EP 2213738 B1	10 October 2012
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		US 2007088153 A1	19 April 2007
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		US 2008091004 A1	17 April 2008
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		US 2011319474 A1	29 December 2011

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/CN2018/104552

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
WO 2015027895 A1	05 March 2015	CN 105473164 A	06 April 2016
		US 2016272967 A1	22 September 2016

REFERENCES CITED IN THE DESCRIPTION

Patent documents cited in the description

- WO 9932619 A, Fire **[0030]**
- WO 9953050 A, Waterhouse **[0030]**
- WO 9961631 A, Heifetz **[0030]**
- WO 0044895 A, Limmer **[0030]**
- DE 10100586, Kreutzer **[0030]**

Non-patent literature cited in the description

- Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, 1982 **[0022]**
- **YANG, D. et al.** *Curr. Biol.,* 2000, vol. 10, 1191-1200 **[0030]**